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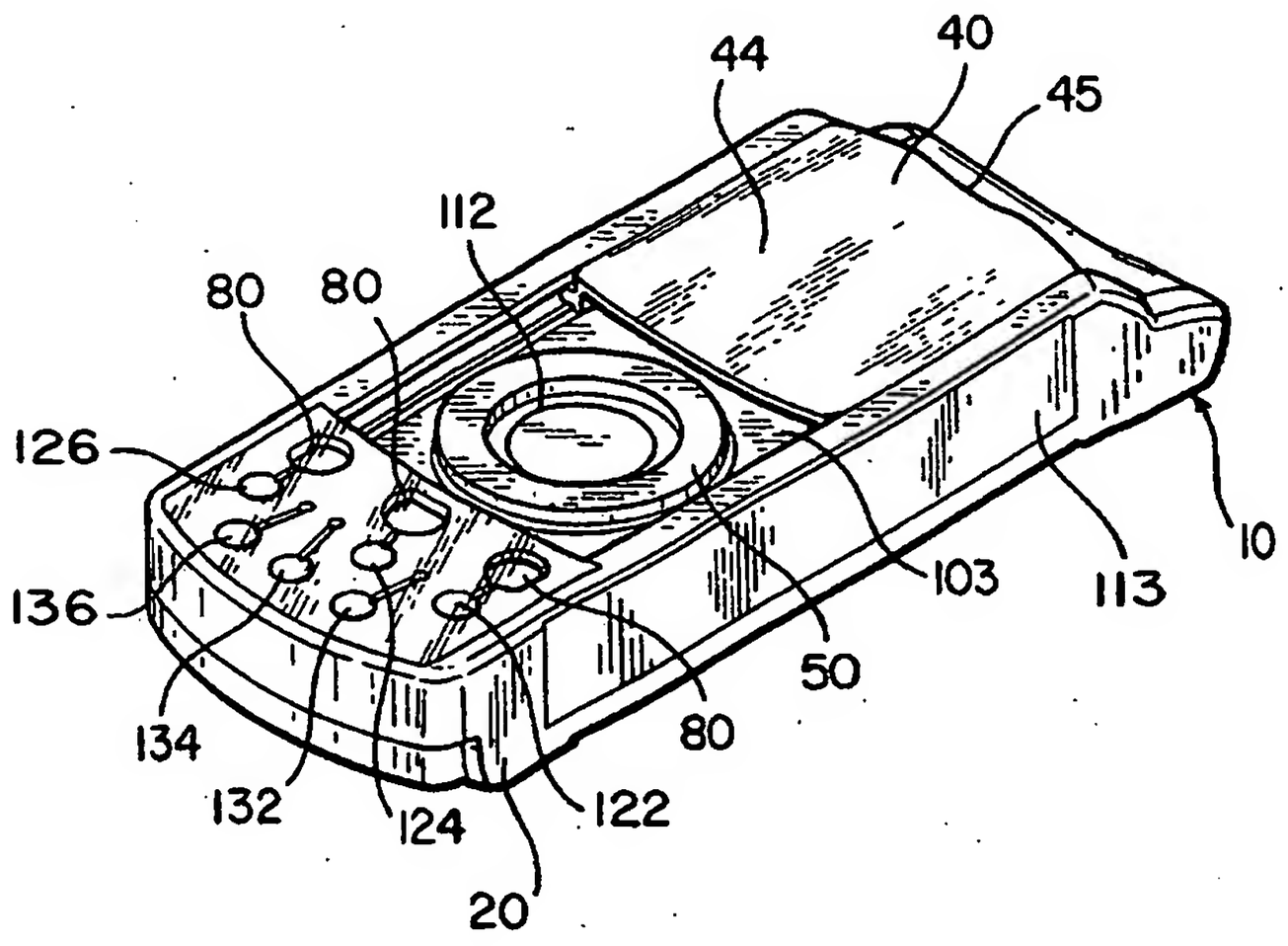

(57) Abstract: A cartridge for receiving an initial volume of liquid, wherein transfer of the liquid to downstream reagent chambers is user controlled. Similarly, the liquid from a reagent chamber may be selectively transferred to a downstream sensing cell which is exposed to a sensing device or analytic device. The cartridge includes an evanescent waveguide optically coupled to the sensing cell.

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CARTRIDGE WITH A SINGLE INLET PORT

This application claims priority of prior provisional application US Serial No. 60/081,677, filed April 14, 1998.

Field of the Invention

The present invention relates to the division of a liquid volume into samples and the presentation of the samples to an analytic device, wherein the samples may be treated prior to exposure to the analytic device.

Background of the Invention

Accurate detection of biological analytes present in various types of test samples is useful for many purposes including clinical, experimental, and epidemiological analyses. Because the genetic information in all living organisms is carried largely in the nucleic acids, either double-stranded deoxyribonucleic acid (DNA) or ribonucleic acid (RNA), detection and discrimination of specific nucleic acid sequences permits the presence, or absence, of a particular analyte within a test sample to be determined.

The collection of blood, the transfer of the blood, the pre-analysis treatment of the blood, the presentation of the blood to an instrument and the disposal of the analyzed blood represent substantial obstacles to the use of blood for analytic purposes. However, as the analysis of blood may provide critical and otherwise unavailable information, blood analysis is carried out in view of the complicated processing. In addition to the inherent logistics in handling blood, as blood may have increased toxicity, exposure of an operator to the blood is also a substantial concern.

Prior blood analysis systems have included collection devices such as the capillary collection device of EP0,545,500 published September 6, 1993. However, the analysis of blood becomes further complicated in the area of determining the presence or amount of antigenic substances in a sample. These determinations are often performed in an immunoassay.

Immunoassay techniques are based on the binding of the antigenic substance being assayed (the "target analyte") and a receptor for the target analyte. Either the target analyte or the receptor may be labeled to permit detection. Various labels have been employed for use in immunoassays, including radioisotopes, enzymes and fluorescent compounds. Many different types of immunoassays are known in the art, including competitive inhibition

assays, sequential addition assays, direct "sandwich" assays, radioallergosorbent assays, radioimmunosorbent assays and enzyme-linked immunosorbent assays.

The basic reaction underlying most immunoassays is the binding of certain substance, termed the "ligand" or "analyte", by a characteristic protein (receptor) to form a macromolecular complex. These binding processes are reversible reactions, and the extent of complex formation for particular analyte and receptor concentrations is regulated by an equilibrium constant according to the law of mass action. Thus, at equilibrium, some of the analyte always exists unbound or "free".

In a competitive inhibition immunoassay, the unknown quantity of target analyte in the sample competes with a known amount of labeled target analyte for a limited number of receptor binding sites. The reagents usually consist of a labeled target analyte, such as an antigen, and a solid phase coupled receptor, such as an antibody. The antigen to be assayed competes with the labeled antigen for binding sites on the coupled antibodies. The concentration of target analyte present in the sample can be determined by measuring the amount of labeled target analyte--either free or bound. This is an indirect assay method where the amount of labeled antigen bound to the antibodies is inversely correlated with the amount of antigen in the test solution. Thus, low concentrations of target analyte in the sample will result in low concentrations of "free" labeled target analyte and high concentrations of "bound" labeled target analyte, and vice versa. The amount of "free" or "bound" labeled target analyte is measured using a suitable detector. Quantitative determinations are made by comparing the measure of labeled target analyte with that obtained for calibrated samples containing known quantities of the target analyte. This method has been applied to the assay of a great number of different polypeptide hormones, enzymes and immunoglobulins.

In sequential addition assays, the reagents used may be the same as in the competitive inhibition assay. However, instead of incubating them at the same time, the unlabeled antigen is first incubated with the antibody, then the labeled antigen is added.

Direct immunoassay systems, also termed "immunometric" assays, employ a labeled receptor (antibody) rather than a labeled analyte (antigen). In these assays the amount of labeled receptor associated with the complex is proportioned to the amount of analyte in the sample. Immunometric assays are well-suited to the detection of antigenic substances which are able to complex with two or more antibodies at the same time. In such "two-site" or "sandwich"

assays, the antigenic substance has two antibodies bound to its surface at different locations. In a typical "forward" sandwich assay, an antibody bound to a solid phase is first contacted with the sample being tested to form a solid phase antibody:antigen complex. After incubation, the solid support is washed to remove the residual sample, including unreacted antigen, if any.

The complex is then reacted with a solution containing a known amount of labeled antibody. After a second incubation to permit the labeled antibody to complex with the antigen bound to the solid support through the unlabeled antibody, the solid support is washed to remove unreacted labeled antibody. The assay can be used as a simple "yes/no" assay to determine whether the antigen is present. Quantitative determinations can be made by comparing the measure of labeled antibody with that of calibrated samples containing known quantities of antigen. "Simultaneous" and "reverse" sandwich assays are also known in the art. A simultaneous assay involves a single incubation step, both the labeled and unlabeled antibodies being added at the same time. A reverse assay involves the addition of labeled antibody followed by addition of unlabeled antibody bound to a suitable solid support. The sandwich technique can also be used to assay antibodies rather than antigens. Such an assay uses as a first receptor an antigen coupled to a solid phase. The antibodies being tested are first bound to the solid phase-coupled antigen. The solid phase is then washed, and then labeled anti-antibody (second receptor) is added.

The above immunoassay methods can be applied to the assay of many different biologically active substances. Among such substances are haptens, hormones, gamma globulin, allergens, viruses, virus subunits, bacteria, toxins such as those associated with tetanus and animal venom, and many drugs. Similar techniques can be used in non-immunological systems with, for example, specific binding proteins.

The assays can be adapted to use a fluorophore. If the properties of the label are not altered by binding, for example, as in a radioimmunoassay, a separation step is required to separate "free" from "bound" labeled target analyte. Such assays, which require a separation step, are called "heterogeneous" assays. If the properties of the label are altered in some way when it is bound, no separation step is required, and the immunoassay is termed "homogeneous."

The measurement of target analytes in biological fluids, such as serum, plasma and whole blood, requires immunoassay methods which are both specific and sensitive. Both the specificity and sensitivity of an immunoassay depend on

the characteristics of the binding interaction between the target analyte and the receptor involved. For example, the reaction must be specific for the analyte to be measured and the receptor used should not bind to any other structurally related compounds. In addition, by choosing a receptor with a high affinity for the target analyte, the sensitivity can be increased.

The label used to monitor the assay may affect the sensitivity of an immunoassay. Labels currently used for immunoassay of target analytes in biological fluids include radioisotopes (radioimmunoassay, RIA), enzymes (enzyme immunoassay, EIA), fluorescent labels (fluorescence immunoassay, FIA), and chemiluminescent labels (chemiluminescent immunoassay, CIA).

FIAs use fluorescent molecules as labels. Fluorescent molecules (fluorophores) are molecules which absorb light at one wavelength and emit light at another wavelength. See Burd, J. F., "Fluoroimmunoassay--Application to Therapeutic Drug Measurement," in P. Moyer et al., Applied Therapeutic Drug Monitoring, American Association of Clinical Chemistry (1984). Typically, an excitation pulse of radiation is directed onto or into a sample, followed by fluorescence of the sample, and the detection of the fluorescence radiation.

FIAs may be either heterogeneous or homogeneous. As noted above, homogeneous assays are usually simpler to perform and are thus, more amenable to automation. However, previously known homogeneous FIAs are less sensitive than heterogeneous FIAs because high background can limit sensitivity. The heterogeneous FIA procedures can detect smaller amounts of analyte than present homogenous FIAs, but only because the separation and washing steps in the assays serve to eliminate background interference from biological substances. In solid phase fluorescent assays the solid support can limit sensitivity at the wavelengths of presently used fluors. In many cases the support itself will fluoresce at wavelengths of commonly used fluors such as fluorescein (493 nm). FIAs also offer the advantage of using stable reagents.

Another assay method uses enzyme-enhanced fluorescence technology which combines microparticle capture and antigen-antibody reaction with an enzyme rate reaction using a fluorescent enzyme substrate. The rate reaction is monitored by steady state fluorometric measurement. In an enzyme-enhanced fluorescence assay, the analyte in question is "captured" by an antibody bound to a solid phase and the solid phase is washed.

An enzyme is then bound to the captured analyte using an enzyme-anti analyte conjugate. Excess reactants are washed away and the amount of enzyme is

measured by the addition of a non-fluorescent substrate. As the enzymatic reaction proceeds, the non-fluorescent substrate is converted to the fluorescent product. Thus, the rate at which the fluorescent product is generated is directly proportional to the concentration of analyte in the test solution.

As discussed above, fluorescence is a phenomenon exhibited by certain substances, which causes them to emit light, usually in the visible range, when radiated by another light source. This is not reflection, but creation of new light.

In most assays using a light measurement it is the intensity of the light that is correlated to drug concentration (so any variations in source light intensity will directly affect the sensitivity of the assay), the sensitivity of FPIAs is independent of intensity variations. Conventional FPIAs require separate measurements of both blank and sample.

Theoretically, fluorometry is capable of being the most sensitive of all analytic tools as it is possible to detect single photon events. A problem which has plagued fluorescence immunoassays has been discriminating the fluorescent signal of interest from background radiation. The intensity of signal from background radiation may be up to 10,000 times larger than the intensity of the fluorescent signal of interest.

The problem of background detection is particularly pronounced in assay of biological samples. Many of the current fluorescence assays use the fluorescent molecule, fluorescein. Fluorescein has an excitation maximum of 493 nm, and there are numerous substances in biological fluids with overlapping excitation and emission similar to fluorescein. For example, in the analysis of blood plasma, the presence of a naturally occurring fluorescable material, biliverdin, causes substantial background radiation. Such compounds are highly fluorescent and contribute significant background signals which interfere with the label's signal, thus limiting the sensitivity of assays using fluorescein labels.

Earlier attempts to overcome the problem of background radiation have met with limited success. One technique for overcoming the problem involves discriminating against background radiation on the basis of wavelength. Filters have been used to reject detected radiation at all but a narrowly defined wavelength band. This technique has been less than successful principally because the background radiation may also be at the same wavelength as the desired fluorescence signal, accordingly, still be passed through the filter and detected.

It has been recognized that for analysis of biological fluids, it would be desirable to use a dye or label which is excitable at radiations of wavelengths of greater than background radiation. However, even though the background fluorescence of serum falls off at wavelengths approaching 600 nm, significant decrease does not occur until 650 nm or greater. Previous attempts to create dyes of such wavelengths have been unsuccessful. See, e.g., Rotenberg, H. and Margarfit, R., Biochem. Journal 229:197 (1985); and D. J. R. Laurence, Biochem. Journal 51:168 (1952).

A second technique attempting to discriminate the desired fluorescent signal from the background is the so called time gating approach. Here, the fluorescent signal is observed in a short time window after the excitation. The time window may be varied both in its length and in its starting time.

Recently, significant advances have been made in the area of fluorescable dyes. In one aspect, dyes being excitable by longer wavelength radiation, such as in the red and infrared wavelengths, are now available.

Despite the significant and promising improvements made in the field of fluorescable dyes, and in the data analysis aspects, the actual methods and apparatus for achieving and detecting fluorescence have heretofore remained relatively unchanged. Utilizing even the most sensitive and best equipment, analysis can take an hour or more, even at high concentrations of materials. When fluorometry is used for immunoassay in a clinical context, time for analysis and proper diagnosis can be absolutely critical. Patient survival can depend on accurate, timely analysis.

Additionally, rapid testing would permit retests of patients without having them wait significant periods of time, resulting in more rapid and accurate diagnosis. As to sensitivity, it is extremely desirable to be able to detect minute amounts of fluorescable material. However, as the amount of fluorescable material in a sample decreases, the ratio of the signal increases. Further, since the time for analysis depends on the amount of fluorescent radiation received from the detector, low concentrations generally require substantially more time to analyze.

The time required for analysis has been prohibitively high. Known methods and apparatus for FIAs have failed to provide rapid and accurate diagnosis and analysis of samples. This has been so despite the clear and known desirability of the use of FIAs. For example, the drug digoxin, which is used to treat congestive heart failure, has a narrow therapeutic range (i.e., serum levels of

0.5 to 2.5 ng/ml and is generally toxic at concentrations greater than 2.1 ng/ml. Present assays using fluorescence-based methodologies require an extracting process to remove interfering substances, such as proteins, in order to detect digoxin at its therapeutic levels. This additional extraction step increases the time, cost and equipment needed to perform the assay.

A crux of the problem relating to the analysis of blood resides in the handling and transport of the blood from the patient to the instruments for analysis. Further, pre-analysis treatment of the blood currently requires individual handling of sample. Such handling is time consuming and expensive. In addition, the pre-analysis treatment often requires mixing of the liquid and reagents or other chemicals.

From the above discussion it can be seen that, although many different types of immunoassays currently exist, none is satisfactory for measuring small quantities of target analytes in biological fluids such as serum, plasma and, especially, whole blood.

The combination of an evanescent waveguide and immunoassay procedures is disclosed in United States Patents No. 5,677,196; 5,512,492 and 5,516,703, and WO 97/35203, each of which is herein incorporated by reference and incorporated as a part of the present disclosure.

Summary of the Invention

The present invention provides a method and apparatus for receiving, dividing, treating and presenting a liquid sample to an analytic device. The invention employs a liquid pathway that may be disposed in a substantially self contained cartridge that can interface with a variety of analytic or sensing devices. The present invention may be incorporated into a cartridge format, wherein a single inlet port is employed and the liquid is distributed to a plurality of downstream reagent chambers and sensing cells. As a plurality of sensing cells may be operably filled from a single common sample, the need for sequential running of independent calibration tests is obviated.

The selective and controllable motion of the liquid within the cartridge and along the pathway can be accomplished by an active pressure induced flow, as well as a combination of capillary and active pressure induced flow.

In the active pressure regime, the device for transferring a liquid to a sensing cell includes: a primary reservoir having an inlet port; a reagent chamber fluidly connected to the primary reservoir; a venting port fluidly connected to the

reagent chamber; a downstream sensing cell fluidly connected to the reagent chamber; and a second venting port fluidly connected to the sensing cell.

In the combined capillary and active pressure regime, the device further includes a well fluidly connected to the primary reservoir; and a foam member disposed in the reagent chamber to expose a portion of the foam member to the well.

The cartridge provides for the selective mixing of chemicals and the liquid introduced into the cartridge. The mixing is controllable and does not require agitation of the cartridge itself. The cartridge format also provides for "point-of-care" analysis of the blood. That is, blood may be promptly introduced into the cartridge and the cartridge placed in a testing device. The testing device may automatically induce transfer of the blood within the cartridge, separate the sample into subvolumes, selectively induce mixing within a given subvolume, selectively transfer a subvolume to a sensing cell and analyze the respective subvolumes.

The present invention may be employed in chemical and especially biological or clinical test procedures, and more particularly, in devices for use with specific binding assay procedures including immunoassay procedures, and particularly those procedures employing an evanescent waveguide. In a particular configuration, the present invention presents a measured and treated sample volume to an evanescent waveguide. In a preferred configuration, the waveguide defines a portion of the liquid pathway.

The present system further allows a controlled residence time of the liquid sample, or a portion thereof, with pre analysis chemicals or treatments. In addition, accurate subvolumes of liquid may be separated from an unmeasured initial sample volume. Thus, the present invention may be employed for a variety of chemical analysis including, but not limited to, DNA, RNA, immunological, clinical chemistry, blood gases, coagulation, hematology, therapeutic drug monitoring and cellular analysis.

Brief Description of the Drawings

Figure 1 is a schematic view of the fluid transfer system.

Figure 2 is a perspective view of a cartridge assembly with the door in an open position.

Figure 3 is an exploded perspective of the components of the cartridge assembly shown in Figure 2.

Figure 4 is a perspective view of the under side of the cartridge top of the cartridge assembly shown in Figures 2 and 3.

Figure 5 is a top plan view of the cartridge top with the door removed.

Figure 5a is a top plan view of an alternate embodiment of a cartridge top with the door removed.

Figure 6 is a cross sectional view taken along lines 6-6 of Figure 5.

Figure 7 is a cross-sectional view similar to Figure 6 for an alternate embodiment.

Figure 8 is a cross sectional view taken along lines 8-8 of Figure 5.

Figure 8a is a cross-sectional view similar to Figure 8 for an alternate embodiment.

Figure 9 is an end view of the cartridge top.

Figure 10 is a side elevational view of the cartridge top.

Figure 11 is a bottom plan view of the cartridge top of Figure 4.

Figure 12 is a schematic cross-sectional view of a waveguide, the cross-sectional view taken along a sensing cell 30.

Figure 13 is a plan view of the upper side of the cartridge bottom according to an alternate embodiment.

Figure 14 is a plan view of the upper side of the cartridge bottom of the cartridge assembly shown in Figures 2 and 3.

Figure 15 is a plan view of the under side of the cartridge bottom.

Figure 16 is a plan view of the intermediate gasket shown in Figures 2 and 3.

Figure 17 is a plan view of the intermediate gasket for the embodiment shown in Figure 13.

Figure 18 is a rear perspective view of a reagent chamber.

Figure 19 is a front perspective view of a reagent chamber shown in Figure 18.

Figure 20 is a cross sectional view of a portion of the cartridge showing liquid in a reagent chamber of Figure 18.

Figure 21 is a cross sectional view of a portion of the cartridge of Figure 20 showing a bubble entering the reagent chamber.

Figure 22 is a cross sectional view of a portion of the cartridge of Figure 20 showing a boundary layer about a bubble in the reagent chamber.

Figure 23 is a cross section view of a portion of the cartridge of Figure 20 showing the travel path of a bubble along the meniscus in the reagent chamber.

Figure 24 is a cross section view of a portion of the cartridge of Figure 20 having a boundary layer surrounding a detached bubble in the reagent chamber.

Figure 25 is a cross section view of a portion of the cartridge of Figure 20 showing a bubble layer in the reagent chamber.

Figure 26 is a cross section view of a portion of a cartridge showing an alternate embodiment of a reagent chamber.

Figures 27-31 are schematic representations of various bubble forming configurations in the reagent chamber.

Detailed Description of the Preferred Embodiments

As shown in the schematic of Figure 1, the present transfer system includes a liquid pathway 6 having a primary reservoir 100 fluidly connected to a downstream reagent chamber 200 through a distribution manifold 150; and a downstream sensing cell 300 fluidly connected to the reagent chamber by a transfer manifold 250. A control pathway 8 is fluidly connected to the liquid pathway 6 through the reagent chambers and sensing cells.

According to various preferred embodiments, the apparatus is embodied in a cartridge assembly, such as cartridge 10 of Figure 2, configured to accommodate any of a variety of testing paradigms and analytes including immunoassays and electrolytes. The cartridge is constructed to enable exposing the sensing cell to a sensing apparatus or analytic device. For those configurations employing evanescent sensing, the cartridge includes an optical waveguide 70 for creating an evanescent wave that penetrates the sensing cell when the waveguide is connected to an evanescent waveguide sensor device.

The cartridge 10 may be constructed to provide a variety of related constructions. For example, a plurality of reagent chambers 200 may be fluidly connected to the primary reservoir 100 through the distribution manifold 150. A corresponding number of sensing cells 300 may be uniquely connected to the corresponding number of reagent chambers 200. Thus, the present invention permits a plurality of liquid samples to be formed from a single common sample source yet treated separately (e.g., treated with unique chemistries), and then presented to the analytic device. As a first example, multiple types of assays can be run from a single common sample and using the same cartridge where the various types of assays are based on the individual reagent chambers. Alternately, one or more of the reagent chambers can be used to calibrate a measuring instrument with another reagent chamber used to run the desired assay, again

using a single common sample and the same cartridge. Alternately, several assays of the same type can be run using a single sample and single cartridge to obtain multiple readings with minimized experimental error.

It is also contemplated that the present invention may be employed with a single reagent chamber 200 and single corresponding sensing cell 300. Alternatively, the invention may be practiced with a single reagent chamber 200 supplying a plurality of sensing cells 300 or a plurality of reagent chambers supplying a lesser number or a single sensing cell.

The cartridge 10 of the present invention may be configured to transfer liquid along the liquid pathway 6 from the primary chamber 100 to the sensing cells 300 in either of two regimes: (a) an active pressure regime and (b) a combined capillary-active pressure regime. As used herein, the term "active pressure regime" and like terms denote that liquid is transferred mainly along the liquid pathway by connection to an external pressure source, whereas the term "combined capillary-active pressure regime" and like terms denote that transfer of liquid relies on both capillary forces on the liquid as well as connection to an external pressure source, i.e., mere connection to an external pressure source would not adequately facilitate liquid transfer through the device.

For purposes of the following description, a cartridge 10 is set forth as including a single primary reservoir 100, three reagent chambers 200 and three corresponding sensing cells 300. However, as discussed above, it is understood the invention is not limited to the single primary reservoir-three reagent chamber-three sensing construction in the illustrated, described embodiments.

Cartridge 10 is constructed of materials and assembled to render the cartridge a single use and disposable device. However, it is understood the cartridge 10 may be formed of appropriate materials to render it reusable according to acceptable medical practices. Additionally, cartridge 10 is preferably sized and shaped to be held in the hand of an operator.

Referring to Figures 2 and 3, the cartridge 10 includes cartridge top 20, a cartridge door 40, a door gasket 50 and pressure source seal 60, a waveguide 70, three reagent chambers 200 (202, 204, 206), an intermediate gasket 310, a cartridge bottom 350, and a channel forming tape 390.

Cartridge Top

Referring to Figures 2-11 the cartridge top 20 includes the primary reservoir 100, three sensing cell windows 30, upper and lower door tracks 22, 24, and a pressure port array 120. Cartridge top 20 may be constructed of a plastic

material, especially a moldable thermoplastic resin such as acrylonitrile-butadiene-styrene (ABS), polypropylene, polyethylene, and the like. Preferably, the materials are formulated to be broad spectrum light absorbing and preferably black. Particularly, the material absorbs the wavelengths employed in an evanescent waveguide configuration.

The three sensing cell windows 30 are located at a first end of the cartridge top 20. The sensing cell windows 30 are arranged adjacent to the sensing cells and provide access from the analytic device to liquid received in the sensing cells. Preferably, the periphery of each sensing cell window 30 substantially corresponds to the periphery of each corresponding sensing cell. For the illustrated embodiment, the lower door tracks 24 extend along the cartridge adjacent the sensing cell windows 30.

Referring to Figures 4, 9 and 11, the cartridge top 20 includes a plurality of spaced teeth 76. The spaces between the teeth are aligned with the sensing cells 30, so that the teeth preclude the passage of exitation light into the waveguide 70.

The pressure port array is located at a second end of the cartridge top 20 and formed in a surface of the cartridge top that is higher in elevation than the surface in which the sensor windows 30 are formed. The pressure port array provides an interface between the control pathway 8 and the exterior of the cartridge 10, wherein the control pathway is connectable to a controllable positive and/or a negative pressure source. The pressure port array 120 preferably includes a first array of three reagent chamber pressure ports 122, 124, 126 and a second array of three sensing cell pressure ports 132, 134, 136. The reagent chamber pressure ports 122, 124, 126 first communicate with the reagent chambers in the liquid pathway 6. The sensing cell pressure ports 132, 134, 136 first communicate with the sensing cells in the liquid pathway 6.

The control pathway 8 is thus defined as extending from the reagent chamber pressure ports 122, 124, 126 to the reagent chambers 200, and from the sensing cell pressure ports 132, 134, 136 to the sensing cells 300.

Each reagent chamber pressure port 122, 124, 126 is separated from and connected to the respective reagent chamber by a pathway, and each sensing cell pressure port 132, 134, 136 is separated from and connected to the respective sensing cell 300 by a pathway. As illustrated in Figure 5, each reagent chamber pressure port 122, 124, 126 is separated from and connected to the respective reagent chamber by a rupture channel 128, and each sensing cell pressure port

132, 134, 136 is separated from the respective sensing cell 300 by a rupture channel 138. The rupture channel 128 has a geometry to reduce the migration of liquid films or bubbles along its length. Thus, the rupture channel inhibits the translation of liquid film or bubbles from a reagent chamber to a respective pressure port. Alternately, as illustrated in Figure 5a, each reagent chamber pressure port 122, 124, 126 is separated from the respective reagent chamber by a rupture channel 128, a well 129, and a second rupture channel 128'. If the mixture of sample liquid and reagent mixed in the reagent chamber has a tendency to foam, the inclusion of well 129, intermediate of the reagent chamber and the reagent chamber pressure port 122, helps to ensure that any foam is entrapped in well 129 and does not reach and contaminate the pressure port. The rupture channels are configured to substantially preclude the formation of capillary films at the reagent chamber and the sensing cell pressure ports.

A pressure source seal 60 is attached to the cartridge top 20 to seal the pressure port array 120. The pressure source seal 60 is selectively puncturable for communicating a negative or positive pressure source with a given pressure port. The pressure source seal may be made of a material such as a sheet of plastic or metal foil, such as aluminum, or combinations thereof, the sheet having a layer of pressure sensitive adhesive for adhering to the uppermost surface of top 20. In a preferred construction, the pressure source seal 60 is a laminate of a polyester film and an adhesive such as 3M-467 adhesive sold by Minnesota Mining & Manufacturing. To reduce possible contamination, the adhesive is cut to a configuration having apertures overlying the pressure port array and rupture channels. Thus, the liquid in the cartridge is not exposed to the adhesive.

The configuration of the pressure ports is at least partially dictated by the construction of the particular pressure source. For example, the pressure ports are arranged, and provide cavities sized and shaped, to receive desired pressure probes, within system tolerances.

The primary reservoir 100 is located in the cartridge top 20 between the sensing cell windows 30 and the pressure port array. For the illustrated embodiment, the upper door tracks 22 extend along the cartridge adjacent the primary reservoir, and the primary reservoir 100 has the general form of a concave recess 102 in surface 103, surface 103 being intermediate in elevation of the surface including the sensing cell windows and the surface including the pressure port array. Reservoir 100 includes a single outlet 104 in a lower portion of the reservoir, with the inlet of the primary reservoir defined by the top of recess

102 in surface 103. The outlet 104 is fluidly connected to the distribution manifold 150. Reservoir 100 may receive liquid from any of a variety of sources such as syringes, pipettes or other aliquoting devices.

The primary reservoir 100 is sized to receive an unmeasured volume of sample liquid such as blood, i.e., it is not necessary to dispense a pre-metered amount of sample liquid to reservoir 100. The unmeasured volume may be any volume within a range. Although the range and hence primary reservoir size are at least partially dictated by the intended uses of the cartridge, it has been found that accommodating a range of between approximately 75 to 900 microliters is desired for immunoassays. According to one configuration, reservoir 100 has a volume to accommodate 500 μL of fluid, and outlet 104 has a diameter of about 0.04 inch.

A critical requirement is that the sample volume in the primary reservoir 100 be greater than a minimum functioning volume of the cartridge. The maximum volume is readily determinable by a liquid level indicator in the primary reservoir 100. The liquid level indicator may be a variety of configurations. For simplicity, the liquid level indicator requires no moving parts or pressure regulation. In the present cartridge 10, the liquid level indicator includes a film or sheet material 112 occluding a portion of the inlet 100. In a preferred configuration, the film 112 is in the shape of an annulus having an outer diameter greater than the perimeter of recess 102 at surface 103 and an inner diameter slightly smaller than this perimeter, wherein the film is concentrically aligned with this perimeter. The film annulus is preferably translucent or transparent so as to permit visual feedback. Other configurations are possible. For example, an alternative configuration of the liquid level indicator, shown in Figure 7, includes an inwardly projecting peripheral rib 114 within the primary reservoir located at an upper portion of recess 102. In either configuration, upon the liquid contacting the liquid level indicator, the surface of the liquid presents a distinctly different reflection of light thereby identifying the desired level of liquid has been received in the primary reservoir 100.

The primary reservoir 100 is configured to substantially preclude capillary retention of the liquid within the primary reservoir. Capillary forces will retain liquid within the primary reservoir 100. Further, the primary reservoir 100 may be configured to provide an employable pressure head within the liquid retained in the primary reservoir. As shown in the accompanying Figures 1-11, the primary reservoir 100 is sized to accommodate a greater volume than the

combined volumes of reagent chambers 200. In a desired construction, the primary reservoir 100 has a volume that is sufficiently greater than the downstream components to minimize subsequent downstream air-liquid interfaces that may alter the properties of the liquid.

The primary reservoir 100 defines a side elevational view that converges from the top to the bottom of the reservoir. As illustrated in the cross-sectional view of Figure 8, reservoir 100 may be shaped as a generally concave well. As illustrated in the cross-sectional view of Figure 8a, reservoir 100 may be shaped as a well having a concave bottom and frustoconical side walls. In the case where the sample fluid has a tendency to foam, such as whole blood, the reservoir configuration shown in Figure 8a may better inhibit the foam reaching the venting port 44 in door 40. More specifically, the foam will tend to adhere surfaces of reservoir 100, and for the embodiment shown in Figure 8a, the foam would tend to follow the side walls. However, it is understood the primary reservoir 100 may be constructed to have alternate shapes, including an asymmetric cross section. Vertical walls in the primary reservoir are believed to assist in draining liquid from the reservoir. Specifically, steeper walls adjacent the outlet 104 enhances the transfer of liquid from the primary reservoir 100.

Chemical agents may be added to the primary reservoir 100, as dictated by the desired intended use of the cartridge. For example, when used with whole blood, it has been found advantageous to employ an antifoaming agent such as "Antifoam A A5758" as distributed by Sigma. The antifoaming agent may be disposed in a portion of the primary reservoir 100 prior to use of the cartridge 10, for example, a patch 101 of the antifoaming agent may be adhered to the surface of reservoir 100, whereby the antifoaming agent dissolves in the sample when added to the primary reservoir.

As shown in Figures 2 and 3, a bar code may be affixed to the cartridge, for example, on side panel 113. The bar code information may include manufacturing data such as lot number, manufacturing location and factory calibration. Alternately, a bar code may be used to log and track the cartridge and sample therein.

The primary reservoir 100 is selectively sealed by the cartridge door 40. The cartridge door 40 is connected to the cartridge top 20 by a first pair of tabs 43 slidably engaged in the lower door tracks 24 and a second pair of tabs 42 slidably engaged in the upper door tracks 22, whereby the door is movable between a closed position and an open position. In the open position, the cartridge door 40

overlies the sensing cell windows 30 and exposes the primary reservoir 100 to receive a liquid sample therein. In the closed position, a seal is formed between the cartridge door 40 and the primary reservoir 100, and the sensing cell windows 30 are exposed. It is understood the cartridge door 40 may be pivotally or hingedly connected to the cartridge top 20. For the illustrated embodiment, however, door 40 is slidable, and a recess 45 may be provided in the edge of the door to guide an operator's thumb when pushing the door into the closed position.

The door gasket 50 is connected to the cartridge top 20 with the film annulus 112 of the liquid level indicator sandwiched therebetween. The door gasket 50 is a resilient seal, such as a closed cell foam or silicone rubber washer concentric with the perimeter of recess 102. The cartridge top 20 and the cartridge door 40 are constructed such that upon disposing the cartridge door in the closed position, the resilient door gasket 50 is slightly compressed. Preferably, the door gasket 50 compression is sufficient to substantially preclude migration of the retained liquid through door gasket under operating conditions. Preferably, the underside of door 40 includes a corresponding raised annulus that contacts and applies pressure about gasket 50 when the door is in the closed position.

In an alternative construction for sealing the primary reservoir 100, as illustrated in Figure 7, the cartridge top 20 may include a peripheral rib 54 projecting upwardly about the perimeter of recess 102 of the primary reservoir. The cartridge door 40 may include a corresponding compressible door gasket connected or bonded to the underside of the cartridge door that aligns with the peripheral rib when the door is in the closed position.

The cartridge door 40 includes a venting port 44 located to be exposed to the primary reservoir 100 in the closed position of the cartridge door. The venting port 44 is constructed to substantially preclude formation of a liquid film across the port, for example, in the case where foaming of the sample liquid contacts port 44. For this purpose, venting port 44 may include a periphery having an acute or right angles, for example, venting port may have the form of a square or rectangular shaped recess. A hydrophobic material may be located over the venting port 44 to inhibit any egress of liquid through the venting port.

The cartridge top 20 also includes wells 80 for receiving the reagent chambers 200. Each well 82, 84, 86 is connected to a corresponding reagent chamber pressure port by a rupture channel 128. Preferably, the wells 80 have an asymmetric cross section for assisting in locating the respective reagent chambers

in a preferred orientation. For the illustrated embodiment, the wells 80 have a generally circular cross-sectional shape with a flat 83 to form the asymmetry. Although each well 80 may have a different size, the illustrated embodiment includes three wells of equal size.

The reagent chambers 200 are separately formed elements and are sized to be received within the wells 80 in the cartridge top 20. The sample liquid is exposed to and mixed with a desired reagent in the reagent chambers. As each reagent chamber 202, 204, 206 is independently formed, the chemistry of the cartridge is readily configurable by mere accommodation of the reagent chamber during formation of the cartridge. In addition; the reagent chambers 200 may be formed of particular colors depending upon the particular chemicals located in the reagent chamber. The reagent chambers 200 may be formed to define different volumes, however, in the illustrated embodiment, each reagent chamber has an equal volume.

Depending upon the intended liquid for the cartridge, the walls of the reagent chamber 200 may be formed of a polymeric material that is relatively wettable or non-wettable. A suitable material is a plastic material, especially a moldable thermoplastic resin such as acrylonitrile-butadiene-styrene (ABS), polypropylene, polyethylene, and the like. The specific grade of plastic resin may be chosen based on a desired wettability for specific applications. In one configuration, the walls of the reagent chambers 200 are formed of a material that is wettable. That is, the reagent chamber 200 is formed of a material that reduces the surface tension of a contacting liquid, facilitating the liquid to spread across, or penetrate, more easily the surface of the walls of the reagent chamber.

The reagent chamber 200 may have a volume from approximately 0.1 to approximately 500 microliters. In one configuration of the reagent chamber 200 for processing blood samples, a volume from 50 to 500 microliters is preferred. As an example, the reagent chamber 200 may have a total volume of approximately 150 μL, for retaining a blood volume of approximately 100 μL. An expected application may employ less than approximately 250 μL of liquid and often approximately 100 μL. Thus, the total volume of the reagent chamber 200 may be less than approximately 250 μL. Further, the surface area of the liquid in the reagent chamber 200 is to be minimized so as to reduce the negative effects of a liquid-air interface.

Waveguide

The waveguide 70 is an evanescent waveguide and is preferably constructed of a transparent, optical plastic having high index of refraction with a low intrinsic fluorescence such as polystyrene, polymethylmethacrylate, polycarbonate, and the like. Waveguide 70 is operably retained by engaging an underside of the cartridge top 20. For example, a pair of tabs 71 on the waveguide may engage with a pair of notched recesses 72 in the bottom of the carriage top, with the distal edge 75 of waveguide 70 being received beneath a pair of projecting tabs 73 on the carriage top. Additionally, edge 74 of the waveguide is adjacent to inner surfaces of teeth projections 76 on the cartridge top.

Light enters waveguide 70 at edge 74 and exits at edge 75, with light being reflected within the waveguide, as schematically illustrated in Figure 12. Thus, edge 75 of the waveguide is formed as an exit aperture to allow light to exit the waveguide, in other words, light exists the waveguide at edge 75 rather than being reflected back in the waveguide. In the present configuration, edge 75 is aligned with aperture 311 in intermediate gasket 310 to facilitate exit of light at edge 75 and absorption of the exited light in the adjacent portion of the cartridge.

In the present configuration, the waveguide 70 is located adjacent the sensing cell windows 30. The cartridge 10 and the waveguide 70 are constructed so that an evanescent wave formed by the waveguide extends downward away from the sensing cell window 30. It is understood an evanescent wave also extends upward away from the waveguide 70.

Typically, waveguide 70, especially in the areas forming the sensing cells, may be coated with a plurality of capture molecules that are immobilized on the surface in contact with the sensing cells. The capture molecules may form in patches as shown in Figure 3. Typical capture molecules include whole antibodies, antibody fragments such as Fab' fragments, peptides, epitopes, membrane receptors, whole antigenic molecules (haptens) or antigenic fragments, oligopeptides, oligonucleotides, mimitopes, nucleic acids, and mixtures and combinations thereof. The capture molecules are generally chosen so that they bind to a binding moiety present on analyte molecules in the sample received in the sensing cells. Evanescent waveguides of this type are fully described in US Patent Nos. 5,512,492 and 5,677,196, and WO 97/35203, the disclosures of which are incorporated herein by reference.

The intermediate gasket 310 is connected to the underside of the cartridge top 20 to dispose the waveguide 70 and the reagent chambers 200 therebetween.

The gasket 310 has a thickness that forms the depth of the sensing cells 300. The gasket 310 includes three sensing cell apertures 313 corresponding to the sensing cell windows 30 in the cartridge top. The gasket 310 also includes three reagent chamber inlet apertures 312, 314, 316 located to align with the reagent chamber inlets. The gasket 310 also includes three reagent chamber outlet apertures 322, 324, 326 aligned with corresponding outlets to the reagent chambers 200. The gasket 310 further includes three sensing cell pressure apertures 332, 334, 336 aligned with the respective sensing cell pressure ports 132, 134, 136. The gasket 310 also includes a primary reservoir outlet aperture 340 aligned with outlet 104 of the primary reservoir. In addition, the gasket 310 may include locating apertures for engaging corresponding locating bosses or tabs on the cartridge top 20. For example, opening 337 may be provided for alignment with a corresponding boss 338 on the bottom of cartridge top 20, in order to facilitate proper alignment of gasket 310 with cartridge top 20 during assembly.

The intermediate gasket 310 may be constructed of a sheet of a material, such as plastic, with both sides of the sheet having a layer of pressure sensitive adhesive for adhering the sheet to the bottom of the cartridge top 20 and the top of the cartridge bottom 350 when sandwiched therebetween. The adhesive should be non interfering with the liquid to be tested or the chemicals employed. An illustrative gasket composition is a laminate of filled black polyester film (such as the product "Questar" available from Film Quest, Chicago, Illinois) intermediate two layers of pressure-sensitive adhesive (such as the adhesive 3M-467 available from Minnesota Mining & Manufacturing). Gasket 310 preferably absorbs a broad spectrum of light and preferably all light, especially the wavelength of any exitation light or anticipated fluorescence. The filled black polyester intermediate film is suitable for this purpose. Preferably, the viable areas for sensing within the sensing cell 300 is slightly less than the entire area of the sensing cell. Thus, the portion of the intermediate gasket nearest the distal end 75 of the waveguide 70 will function to attenuate any light and reduce reflection back into the sensing cell. The gasket may be initially supplied as a laminate of adhesive backed plastic lacking any apertures therein. In forming the various apertures in the intermediate gasket, it is desirable to ensure that minimal debris results from the cutting operation. For this purpose, the various apertures may be formed in the initial plastic sheet with a laser or die cut.

Figure 17 illustrates an alternate embodiment of an intermediate gasket 310 adapted for the cartridge bottom shown in Figure 13. Gasket 310 includes apertures 320 aligned with corresponding recessed regions 370.

Referring to Figures 13-15, the cartridge bottom 350 is connected to the bottom of the cartridge top 20 to dispose the waveguide 70, the reagent chambers 200 and the intermediate gasket 310 therebetween. Cartridge bottom 350 may be formed of similar materials as the cartridge top.

The sensing cells 300 are thus defined by the waveguide 70, the intermediate gasket 310 and the top side of the cartridge bottom 350. Each sensing cell 300 has an inlet at the sensing cell inlet through hole in the cartridge bottom 350 and an outlet at the sensing cell outlet through hole in the cartridge bottom 350. Each sensing cell is sized to retain a volume of liquid that is less than the volume of liquid retained in the corresponding reagent chamber 200. The sensing cell 300 is located beneath the waveguide 70. The materials of the cartridge bottom 350 and intermediate gasket 310 are selected to absorb light, the sensing cell 300 is substantially defined by a portion of the waveguide 70 and black materials.

Referring to Figure 12, a portion 310' of the gasket 310 is located with respect to waveguide 70 opposite the point of first internal reflection in the waveguide. The light absorbing characteristic of the gasket 310 reduces light contamination in the corresponding sensing cell.

The cartridge bottom 350 includes a top side and a bottom side. The top side defines the distribution manifold 150 extending between the primary reservoir 100 and the reagent chambers 200. More specifically, the distribution manifold 150 is formed in the top side of the cartridge bottom 350 by a recessed region in this top side and extends from the area of the primary reservoir outlet aperture 340 in the intermediate gasket 310 to the reagent chamber inlet apertures 312, 314, 316 in the intermediate gasket. The distribution manifold 150 includes grooves 151, 152, 153 recessed in the top side of the cartridge bottom and emanating from a recessed region 154 in the area of, and in communication with, the primary reservoir outlet. As shown in the embodiment illustrated in Figures 3 and 15, these grooves extend from recessed region 154 and terminate at a location aligned specifically with the reagent chamber inlet. Alternately, as shown in Figure 13, these grooves extend from recessed region 154 to recessed regions 370 surrounding each opening 352, 354, 356. The various recesses and recessed grooves are formed to have sufficient tolerances to accommodate manufacturing

tolerances in the cartridge top 20, the intermediate gasket 310 and the cartridge bottom 350. Accordingly, the lower surface of intermediate gasket 310 applied to this top side of the cartridge bottom, along with the recessed areas and grooves in this top side of the cartridge bottom, forms the discrete pathways separating and linking the primary reservoir 100 and the individual reagent chambers 200. According to one configuration, recess 154 may have a height of about 0.02 inch and a width of about 0.14 inch; grooves 151, 152, 153 may have a height of about 0.2 inch and a width of about 0.04 inch.

The distribution manifold 150 may have alternate configurations that allow liquid passage between the primary reservoir and each reagent chamber. For example, the distribution manifold 150 may include a single duct extending from the primary reservoir 100, wherein the single duct terminates at a hub and individual branches extend from the hub to a corresponding reagent chamber. Although each branch may be constructed to exhibit a particular resistance to flow, as dictated by cartridge configuration, it is more typical that substantially equal flow resistance occurs among the branches.

According to certain embodiments of the invention, the top of the cartridge bottom may include projecting stems 351 for each opening 352, 354, 356, stems 351 being raised from the top surface of the cartridge bottom to be received in the reagent chamber outlet opening, with openings 352, 354, 356 extending as through holes in the stems. The pair of recesses 74 on the top of cartridge bottom form no part of the transfer manifold but instead are included on the illustrated embodiment to receive projecting tabs 73 of the cartridge top.

The bottom side of the cartridge bottom 350, in conjunction with the tape 390, generally defines the transfer manifold 250 and the control pathway 8 from each sensing cell 300 to the respective sensing cell pressure port. The transfer manifold 250 is formed as a recessed area on the bottom side of the cartridge bottom 350 and extends from the reagent chamber outlets 352, 354, 356 to the sensing cell inlet through hole 258, 260, 262. The control pathway 8 extends as a recessed area from the sensing cell outlet through holes 264, 266, 268 to the sensing cell pressure port through holes 362, 364, 366. More specifically, these recessed areas may have the form of grooves recessed in the bottom side of the cartridge bottom, the grooves extending between the desired ports and through-holes. A channel forming tape 390 is applied to the bottom side of the cartridge bottom 350. Accordingly, these grooves and the surface of the channel forming tape form the discrete pathways that fully define the transfer manifold 250 and the

control pathways from the sensing cell 300. According to one configuration, the grooves in the bottom side of the cartridge bottom may have a height of about 0.02 inch and a width of about 0.05 inch; the orifices linked by these grooves may have a diameter of about 0.03 inch. According to this configuration, the sensing cells may have a height of about 0.13 inch, a width of about 0.24 inch, and a volume of about 60 μL.

Channel forming tape 390 may be constructed with a material that provides a wetting surface, such as polyester or other wettable plastics. A pressure-sensitive adhesive layer on tape 390, such as 3M-467 adhesive supplied by Minnesota Mining & Manufacturing, may be used to adhere tape 390 to the bottom side of bottom cartridge 350, the adhesive from the tape 390 being non-interfering with the liquid to be tested or the chemicals employed.

Accordingly, the outlet port of each reagent chamber 200 is fluidly connected to a transfer manifold 250 that extends to a sensing cell 300. Although the transfer manifold 250 may connect a plurality of sensing cell to a given reagent chamber, or a plurality of reagent chamber 200 to a given sensing cell 300, it is anticipated that the transfer manifold will be in the configuration of a transfer channel that uniquely connects a given reagent chamber 200 to a single given sensing cell 300.

Thus, the sensing cell 300 locates the liquid in contact with the waveguide 70. The sensing cell 300 may include additional chemical constituents and analytes necessary for the particular analysis such as assays and particularly immunoassays.

The sensing cell 300 is oriented with respect to the evanescent waveguide 70 such that gravity tends to pull the liquid and any suspended particulate matter away from the surface of the waveguide. That is, the sensing cell 300 is below the waveguide 70 and a measured signal is measured from above the waveguide, through the windows 30. It is understood the sensing cell 300 may be configured to locate the liquid above the waveguide 70, so that gravitational forces tend to urge negatively buoyant particulate matter in the liquid to the surface of the waveguide where contact of such particulate matter with the waveguide is desired..

In a desired configuration, the control path 8 connecting the sensing cell outlet to the corresponding sensing cell pressure port has a sufficient volume to accommodate liquid overfill of the sensing cell 300 without contaminating other sensing cells. Thus, the volume of the transfer manifold 250, the sensing cell 300

and the control pathway 8 from the sensing cell outlet to the corresponding pressure port is greater than the volume of liquid available to be drawn from the reagent chamber 200.

It is also contemplated that a sensing cell 300 may include a flow resistor. The flow resistor is employed to increase flow resistance upon a filling of the sensing cell to a predetermined amount or predetermined percentage. The flow resistor may be in the form of a foam barrier adjacent the venting port and spaced from the inlet, so that as liquid travels along the sensing cell 300 the resistance to flow is relatively low, until the liquid encounters the foam and the flow resistance then increases. It is further contemplated the flow resistance may be increased gradually by varying the construction of the foam. Alternatively, the flow resistor may be an intermediate change in the flow path geometry, such as a reduced aperture or tortuous section.

A pressure generator may be selectively connected to the pressure port array 120 to expose the reagent chambers 200 and the sensing cells 300 to a positive or negative pressure. The pressure generator may be one of a variety of constructions such as individual plungers, interconnected plungers or remotely actuated pneumatic systems.

Preferably, the primary reservoir 100, the distribution manifold 150, the reagent chambers 200, the transfer manifold 250, the sensing cells 300 and the control pathway 8 from the sensing cell outlet to the respective sensing cell pressure port are sized so that the volume of any air entrained in the flow pathway or venting paths is sufficiently small so as to be incompressible. Typical volumes are less than 2,000 μL have proved sufficient with volumes of approximately 1,000 μL preferred. Thus, an change in volume of air in the control pathway results in a corresponding movement of an equal volume of liquid in the cartridge.

If desired, the sensing cells or the reagent chambers may be subjected to localized heating or cooling; for example, if localized heating of sample in the sensing cell is desired, the sensing cell and sampling be subjected to a source of infrared radiation.

Referring to Figures 18, 19 and 20, one embodiment of a mixing and reagent chamber 200 is illustrated. The intermediate gasket shown in Figures 3 and 16, and the cartridge bottom shown in Figures 3 and 14, are adapted for use with this embodiment of the reagent chamber. Each reagent chamber 200 includes an inlet 212 and an outlet 214. Flat 283 on the exterior of the reagent chamber sidewall corresponds to flat 83 of well 80. The outlet 214 is located in

the bottom of the reagent chamber 200; as shown in the figures for the illustrated embodiment, outlet 214 is centered with respect to the chamber bottom. The inlet 212 includes an opening in the exterior of, and offset from the center of, the chamber bottom that it is connected to manifold 150. The inlet extends through the chamber sidewall 213 to the chamber interior such that the interior opening is located inside the chamber intermediate the closed end and the opposed open end 215. The reagent chamber 200 may be any of a variety of shapes so long as it is received in corresponding shaped well 80. The chamber bottom may also be a variety of configurations. In a preferred construction, the chamber bottom is configured to minimize areas that inhibit flow or create stagnation zones. That is, the bottom is a continuous surface. In a preferred configuration, the bottom is generally concave and forms a hemispherical surface.

The side walls extend upwardly from the bottom. The side wall is generally vertical, but may be slightly tapered to either a smaller or a larger diameter than the bottom wall. As illustrated in Figure 20, the inlet port 212 for introducing a liquid into the reagent chamber 200 is formed at a juncture of the side wall 213 and the chamber bottom. Preferably, the side wall is contiguous with the bottom and may be part of an integral structure. According to one configuration, chamber 200 has a volume of 150 µL, side walls 213 has a diameter of about 0.25 inch, inlet 212 has a diameter of about 0.04 inch, and outlet 214 has a diameter of about 0.03 inch.

In some configurations, the reagent chamber may also include a top wall 218 (for example, as illustrated in Figure 26). In such configurations employing a top wall, the side wall extends between the top wall and the chamber bottom. However, it is understood the invention may be practiced without requiring a top wall. Although the top wall may be any of a variety of configurations, the top wall 218 is preferably a planar member for precluding the passage of liquids. The top wall may include the vent port 219 fluidly connected to the respective reagent chamber pressure port 122, 124, 126 through rupture channel 128. In configurations lacking a top wall, the reagent chamber is fluidly connected to the respective reagent chamber pressure port and rupture channel by its open end 215. Preferably, the top wall is spaced from the intended fluid height in the reagent chamber 200 to define an ullage volume of approximately 50% of the retained liquid.

The reagent chamber 200 may include a reagent composition 225 that is to be mixed with the intended sample liquid. The reagent may be in the form of a

solid that is to be dispersed through the liquid or reconstituted by the liquid. The reagent chamber 200 may be pretreated with any of a variety of chemicals, or reagents such as anticoagulants, tracers, antigens, antifoaming agents, lysing agents, surfactants, buffers or any combination thereof. Therefore, the reagent chamber 200 may be employed to alter or treat the liquid sample retained therein prior to introducing the sample to the sensor cell. Preferably, these chemicals are installed in a dehydrated state or other state offering enhanced shelf life of the chemical and hence cartridge. It is contemplated that the reagent chamber 200 may be color coded according to a particular parameter of the reagent chamber such as the retained chemicals or volume of the reagent chamber.

An alternate embodiment of a reagent chamber 200 is illustrated in Figure 26. The intermediate gasket shown in Figure 17, and the cartridge bottom shown in Figure 13a, are adapted for use with this embodiment of the reagent chamber This embodiment is discussed below in conjunction with the combined capillary-active pressure regime.

Active Pressure Regime

Referring to Figures 18, 19 and 20, for the active pressure regime, the reagent chamber 200 preferably includes a concave bottom with substantially cylindrical interior walls. Additionally, for this regime, the outlet 214 preferably includes a bubble forming stand pipe 216 extending into the reagent chamber. By introducing gas through stand pipe 216, liquid sample supplied to the chamber from manifold 150 is mixed with a desired reagent present in the reagent chamber prior to introduction of the sample liquid to the sensing cell. In other words, for the active pressure regime, the liquid sample and the desired reagent may be subjected to an active mixing stage to ensure a desired level of mixing of these components is achieved prior to introducing the liquid sample treated with the reagent to the sensing cell.

The bubble forming standpipe 216 is fluidly connected to the transfer manifold 250 and selectively connected to a pressurized gas source. A bubble orifice 217 is located at a terminal end of the standpipe 216 so as to space the bubble orifice from a local area of the bottom wall. Essentially, for the active pressure regime, the orifice in the bubble forming standpipe 216 corresponds to the reaction chamber outlet 214.

The bubble formation system includes the bubble orifice, the control pathway 8 and a pressurized gas source. The bubble orifice 217 is connected to

the transfer manifold 250 and the control pathway 8, wherein the control pathway is connected to the pressurized gas source.

The bubble orifice 217 is formed in the reagent chamber 200 for admitting pressurized gas into the liquid retained in the reagent chamber 200. In a preferred configuration, the bubble orifice 217 is spaced from the reaction chamber bottom and preferably in the center of the bottom wall, so as to be uniformly spaced from the side wall. The standpipe 216 offsets the bubble orifice 217 from the bottom wall so that a bubble formed at the bubble orifice is slightly spaced from the bottom wall. Various configurations of the standpipe and bubble orifice are illustrated schematically in Figures 27-31.

In a first configuration of the invention, the reagent chamber 200 defines a volume symmetrical about a vertical longitudinal axis, wherein the bubble orifice 217 is located on the longitudinal axis. Preferably, the reagent chamber 200 has a generally cylindrical shape about the longitudinal axis such that the bubble orifice 217 is spaced from the bottom wall and located on the longitudinal axis. Further, in a first configuration, the reagent chamber 200 is configured so that the depth of an operable volume of liquid in the mixing chamber is at least .75 the width of the fluid with a more preferred ratio between 1.0 to 1.5.

The bubble orifice 217 may be any of a variety of configurations. For the configuration of the mixing chamber selected to retain approximately 80 µL to 140 µL, the bubble orifice 217 and standpipe 216 has a preferred inner diameter between approximately 0.015 inches and approximately 0.075 inches with an outer diameter between approximately 0.020 inches and 0.080 inches. A preferred inner diameter is between approximately 0.020 inches and 0.040 inches, with a corresponding outer diameter between approximately 0.025 inches and approximately 0.080 inches. The height of the bubble orifice from the adjacent bottom wall is between approximately 0.005 inches and 0.125 inches, with a preferred height of approximately 0.010 inches and 0.100 inches. It is understood these dimensions may be adjusted as dictated by the liquids to the mixed, the available mixing time and volume to be mixed.

The pressurized gas source may be any of a variety of devices including air pumps or containerized pressure containers. Preferably, the gas source is controllable and metered, so that a predetermined flow rate of gas may be presented to the bubble orifice 217. The present system does not require an intermittent metered delivery of gas to the bubble orifice 217, but rather employs a continuous flow from the source to the bubble orifice. The gas may be air, or an

inert gas such as nitrogen if it is desired to deoxygenate the sample in the reagent chamber for anaerobic analysis.

In one configuration, the reagent chamber 200 is constructed so that a bubble introduced by the bubble forming system passes vertically through the entire volume of liquid retained in the reagent chamber 200. That is, the bubble does not generally contact the side wall during an ascension through the retained liquid. This configuration, as well as alternate configurations, are illustrated schematically in Figures 21-25. Bubble forming devices for agitating a liquid sample with a reagent are described more fully in commonly assigned US patent application Serial No. 09/151,559, filed September 11, 1998 and entitled "Method and Apparatus for Selectively Agitating a Liquid", the entire disclosure of which is incorporated herein by reference. The operation of the active mixing stage is discussed in more detail below.

Combined capillary-active pressure regime

Referring to Figure 26, in the combined capillary-active pressure regime, the outlet port 214 in the reagent chamber is preferably located at the terminus of projecting stem 351 within the interior of the reagent chamber 200. Accordingly, the outlet port 214 may be located above the bottom of the reagent chamber 200 and above a peripheral well formed by recessed space 370 in the cartridge bottom.

Although the reagent chamber 200 may be any of a variety of configurations including cylindrical or frustoconical, in one configuration in the capillary-active pressure regime, the reagent chamber is frustoconical with the smallest diameter of the reagent chamber being adjacent the peripheral well at the bottom or lower portion of the reagent chamber 200.

In the capillary-active pressure regime, an open cell foam body 220 is disposed in each reagent chamber 200. A lower portion of the foam body 220 is exposed to the peripheral well and the upper portion of the foam body is spaced from an upper portion of the reagent chamber 200. Thus, the foam body 220 is exposed to the peripheral well at recessed space 370 via opening 320 in the intermediate gasket, spaced from the top of the reagent chamber and surrounding projecting stem 351 defining the outlet of the reagent chamber.

In one configuration of the foam body 220, the foam material has a favorable wetting to aqueous solutions, a high void volume and regular interstices. An available foam is Melamine foam.

In accordance with the invention, foam body 220 may be any compressible solid material which is (a) wetted by the liquid to be collected, and

(b) has a porosity and interconnections between the cells of the foam which are sufficient to provide a capillary influx of liquid placed at the peripheral well, which exceeds that which occurs in the absence of the foam. Compressible open-cell foam are suitable to provide these properties, such as foam the cells of which form an isotropic network, when uncompressed, of very fine filaments of generally uniform thickness and of dimensions and void volume described below.

The foam is a purely skeletal, three-dimensional, network formed without membranes in the network interstices, and hence with no "windows." The three-dimensionally interconnected skeletal filaments of material provide and maintain uniform interstices to improve blood storage and delivery. In contrast to foams which are reticulated by burning techniques, the material of the present invention provide a significantly higher void volume, with no blocked, or partially blocked interstices and have no residual "burned-cell-wall" debris. This results in significant improvements as to the amount of blood which is storable in, and deliverable from given volumes of materials of the present invention.

The interconnecting filaments have a relatively large length to width ratio, for example, in the order of 10 to 1 greater, and preferably are not thicker than 151μm. The innate skeletal nature of foam-type masses of such a foam material yields a low bulk density and large void volume because of the high percentage of their volume comprising interstitial voids. In these aspects, the materials can be characterized as having in their utilized condition (that is uncompressed or compressed condition):

(i) a void volume greater than 90%, preferably greater than 95% and most preferably greater than 98%; or

(ii) a bulk density less than 24kg/m3 (1.5lbs/m^3), preferably less than 16kg/m^3 (1.0lbs/m^3).

In regard to cell size, uncompressed materials can be characterized as having a relatively uniform cell size, with a relatively small percentage of voids significantly smaller than the average cell size. More particularly the materials can be characterized as having:

(i) an average cell size in the range of 25μm to 200μm, one configuration in the range of 50μm to 175μm, and another configuration having a majority of the cells in the size range of 140μm to 160μm; and

(ii) a cell size uniformity such that at least 95% of the cells have a size larger than 0.67 times the average cell size, one configuration of at least 97.5% or the cells have a size larger than 0.67 times the average cell size

and another configuration having at least 99.5% of the cells have a size large than 0.67 times the average cell size.

In regard to composition of foam materials, it is preferred that such materials be relatively inert vis a-vis the liquid, and not swell nor leach.

One highly preferred group of materials, with respect to their composition as well as other aspects described above, are the thermoset foam materials described in US-A-4,929,969 comprising preferably more than 80% melamine-formaldehyde condensate.

This particularly preferred group of melamine formaldehyde condensate foams comprise a plurality of mutually connected, three-dimensionally branched webs (or filaments). As described in US A-4,929,969 the foam structures desirably have:

1. a mean length to width (diameter) ratio in order of 10:1 or greater; and
2. a web or filament density in the order of 1100kg/m3 (1.10g/ml) or greater.

A foam material is "wettable" by the liquid being collected if that material (preferably when unfoamed) forms a contact angle with that liquid that is less than 90°.

Plastic foams which are "non-wettable" by the transported liquid as that term is defined herein, include polystyrene, polyethylene, polypropylene, some poly(vinyl chlorides), and thermoplastic poly ester.

Also as used herein, the "substantial releasing" of the liquid from the material means, the release of at least 85% of the volume of the liquid contained in the foam material when the liquid is subjected to a controlled application of a partial vacuum or a partial pressure different from ambient conditions. A "controlled application" means, at a gradual rate, for example, between 1.0µl/s and 20µl/s. That is, the foam must readily release the liquid collected, for further use (for example, blood plasma released for diagnostic testing.)

Melamine foams of the kind described above are manufactured by BASF Aktiengesellschaft, Federal Republic of Germany (BASF Corp., Chemical Div., Parsippany, New Jersey), and sold under the tradename BASOTECT® by that company or under the tradename WILTEC® by Illbruck Schaumstofflechnik, Leverkusen, West Germany (Illbruck USA, Minneapolis, Minn.). These foams are commonly marketed for heat and sound insulation in buildings, vehicles and larger containers. Other known uses are as shock absorption packaging,

bandages. cleaning materials and soil treatment, or as in US-A-4 929 969, storage of ink.

The foam can be confined within the reagent chamber either substantially uncompressed in all three orthogonal directions, or compressed in any one or all three directions.

It is believed there is a maximum compression percentage beyond which the fill rate will drop below the fill rate available without foam, when the cells become so closed off as to hinder flow. Although that maximum has not been determined, it is believe it is not reached until well beyond 60% compression, considering the 95% void volume provided by the preferred foam).

It is also considered that when the foam is compressed by at least 2%, there is an increase in the fill rate over a zero % compression. The exact mechanism for this increased fill rate due to the foam compression is not understood. It may arise from the fact that the cell compression occurs more at the solid surface of the walls, and that the compression draws the liquid into better contact with the wall surface than would be the case in the absence of any foam whatsoever.

Preferably, the foam body 220 is subject to a compressive force such that at least a portion of the foam body is constricted. The foam body 220 is located within the reagent chamber 200 to substantially surround the outlet. Further, the foam body 220 is sufficiently spaced from the venting port to define an ullage space therebetween. The foam body 220 may be any of a variety of configurations to provide that spaced apart locations of the foam body 220 are subject to different compressive forces. For example, if the reagent chamber 200 is generally cylindrical, the foam body 220 may by constructed as a conical section having a minimum diameter, when uncompressed, that is larger than the smallest diameter of the reagent chamber. Conversely, if the reagent chamber 200 is frustoconic, then the foam body 220 may have a cylindrical shape with a diameter, when uncompressed, at least as large as the widest portion of the reagent chamber into which it is to be located.

The foam body 220 may be pretreated with any of a variety of chemicals, or reagents such as anticoagulants, tracers, antigens, antifoaming agents, lysing agents, surfactants, buffers or any combination thereof. The foam body 220 may be pretreated by a variety of ways such as immersion and drying, vapor deposition or even creation of the foam structure to include the desired chemicals for release upon exposure to the liquid. Thus, the foam body functions both as an attraction

media, to draw sample liquid into the reagent chamber, and a mixing media, to mix the sample liquid with a desired reagent in the foam. In addition, the foam body may be formed to have a particular color depending upon its intended use or chemical treatment.

Further, the foam body 220 is a open cell foam through which the liquid may pass through in a tortuous path. The foam body 220 defines an interstitial or void volume.

The plurality of reagent chambers 200 and corresponding foam bodies may have substantially equal volumes, or the reagent chambers and associated foam bodies may have of differing volumes. As discussed infra, as the reagent chamber 200 and foam body 220 are selected to draw and retain only a predetermined maximum volume of liquid, the reagent chambers and foam body may be selected to retain different volumes in different reagent chambers.

As stated, each reagent chamber 200 is formed of a material exhibiting a wetting surface to enhance the uptake volume of liquid into the chamber from the peripheral well enhance the speed of the liquid uptake. The compression of the foam body 220 adjacent the peripheral well also assists in the intake of the liquid into the interstitial volume of the foam body.

In the capillary-active pressure paradigm, the distribution manifold 150 is sized to enhance capillary flow of the intended liquid between the primary reservoir and the respective reagent chamber. Thus, the distribution manifold 150 has a cross sectional area that is sufficient to at least induce capillary transfer from the primary reservoir through the distribution manifold to the respective reagent chamber.

In the capillary-active pressure paradigm, the distribution manifold 150 at least partially defines a peripheral well defined by recessed space 370 that is exposed to the foam body 220 in the reagent chamber 200 via opening 314 in gasket 310, thereby defining inlet 212 to the reagent chamber. The projecting stem 351 is employed to locate an outlet 214 to the reagent chamber 200 within the foam body 220 in the reagent chamber 200.

Operation

In the initial mode, the reagent pressure ports and the sensing cell 300 pressure ports are closed. That is, the pressure source seal 60 is effectively unbroken for each member of the pressure port array 120. In addition, as shown in Figure 2, the cartridge door 40 is in the open position exposing the primary reservoir 100 and covering and protecting the sensing windows 30.

A sample liquid such as whole blood is introduced into the primary reservoir 100. The primary reservoir 100 is sized to receive an unmeasured volume of blood having a volume that is within the relatively broad range of 75 to 900 microliters.

The cartridge door 40 is then moved from the open position to the closed position so that the door gasket 50 is partially compressed and the liquid is retained in the primary reservoir 100. As the cartridge door 44 is closed, air is self vented through the venting port 44.

Upon disposing the liquid in the primary reservoir 100 and with the pressure port array 120 remaining sealed, air is now trapped within the various pathways of the cartridge; this air is essentially incompressible, and the sample liquid in the primary reservoir will tend to remain within the primary reservoir, with no or little downstream flow toward the reagent chambers 200. As there is no movement of the air, the entrapped air adjacent the reservoir outlet 104 becomes a saturated vapor due to the pressure exerted thereon by the sample liquid in the primary reservoir. The formation and retention of a buffer of saturated vapor adjacent the air/blood interface prevents a sample liquid such as blood, which has a tendency to denature very quickly in ambient environment, from drying and clogging the opening 104. In fact, since the sample liquid is exposed to ambient air only via the port 44 at this stage of operation and entrapped air in the cartridge, denaturing of blood sample is essentially limited to the upper region of reservoir 100. And since the port 44 is remote from the outlet 104 and the reservoir 100 includes a volume of sample in excess of that transferred to the reagent chambers, it has been found that blood samples may be stored in the closed cartridge for over one hour before introduction to the reagent chambers. This is in contrast to prior blood analyses methods where blood samples must be quickly added to a reagent, or otherwise pretreated, to prevent denaturing. In addition, after a period of time during which sedimentation may occur, the cartridge may be initialized by introducing gas or air into the primary reservoir through the primary reservoir outlet. As the gas ascends through the liquid in the primary reservoir outlet, the particulate matter is homogeneously distributed throughout the liquid.

In the active pressure regime, the reagent chamber pressure ports 122, 124, 126 and the sensing cell pressure ports 132, 134, 136 are selectively opened/closed, i.e., the seal is broken on selected pressure ports to thereby introduce a pressure differential that induces liquid flow from reservoir 6 along

the liquid pathway 6. For example, with reference to the reagent chamber 200 located in well 82, the corresponding sensing cell pressure port 132 remains closed, and the reagent chamber pressure port 122 is exposed to a negative pressure source. By drawing a predetermined volume of air through the control pathway 8 from the reagent chamber pressure port 122, a predetermined volume of sample liquid may be drawn from the primary reservoir 100 to the reagent chamber 200 in well 82 (the primary reservoir vent port 44 permitting liquid to exit the primary reservoir). Specifically, sample liquid is drawn from the primary reservoir through outlet 104 to manifold 150, travels through channel 151 and is drawn into the chamber in well 82 through its inlet 212. As the volume of the distribution manifold 150 is known, an appropriate volume of air is drawn from the reagent pressure port 122 and the liquid is transferred to the reagent chamber 200. As the remaining sensing cell pressure ports and reagent pressure ports are closed, no liquid is drawn to the other reagent chambers.

In the case where it is desired to introduce sample liquid into additional reagent chambers, the process may be repeated, in which case the pressure source to well 82 would remain shut off and in place in inlet 212 to effect a seal with respect to the reagent chamber in well 82. Alternately, it is possible to fill several reagent chambers concurrently, provided that the various pressure sources are controlled accordingly. Since each reagent chamber 200 has a unique reagent chamber pressure port, the reagent chambers may be filled simultaneously, or sequentially in any order. Thus, differing required residence times of the liquid in a given reagent chamber may be accommodated by staggering the introduction of the liquid into the respective reagent chamber. In all cases, the volume of liquid introduced into each reagent chamber may be accurately accomplished from a single source of sample liquid.

Optionally, if desired, a positive pressure source may be introduced at one of ports 122, 124, 126, prior to exposing these ports to the negative pressure source to draw sample liquid from reservoir 100. Upon introducing a small volume of gas at these ports, the gas travels to opening 104. This operation may be useful if the sample liquid contains particulates that may have settled in opening 104, whereby the introduced gas tends to disperse the particulates within the sample liquid and preventing clogging or inhibited flow of sample liquid through opening 104.

In the capillary-active pressure regime, when it is desired to transfer liquid from the primary reservoir 100 to the reagent chambers, the pressure source seal

associated with the relevant reagent chamber is ruptured or otherwise opened. For example, in the case where it is desired to transfer sample liquid to a reagent chamber in well 82, pressure source seal is opened. Upon opening this pressure port, the pressure head in the primary reservoir 100 and the capillary action of the distribution manifold 150 induces liquid flow from the primary reservoir to the peripheral well 370 of the reagent chamber 200. However, it is understood that a positive pressure could be applied to the liquid in the primary reservoir to induce liquid flow from the primary reservoir to the reagent chamber, for example, by introducing a positive pressure through port 44. Alternatively, a negative pressure may be applied to the reagent chamber pressure port to assist in drawing the liquid from the primary reservoir 100 to the reagent chamber 200, for example, by introducing a negative pressure through port 122.

Upon the liquid entering the peripheral well of recessed area 370, the liquid contacts the partially compressed foam body 220 in the reagent chamber. The foam body 220, and particularly the compressed portion of the foam body, draws the liquid into the interstitial space of the foam body.

The characteristics of the foam body 220 and the wettable versus non-wettable nature of the reagent chamber 200 are such that the liquid will be readily drawn into the reagent chamber 200 and fill the available interstitial, or void, volume of the respective foam body 220. The foam body 220 and liquid parameters are such that liquid will not be drawn into a reagent chamber 200 beyond the available interstitial space in the foam body 220. The ullage space between the top of the foam body 220 and the venting port will not be filled with liquid. Thus, the reagent chamber 200 and the corresponding foam body 220 draw and retain only a predetermined maximum volume of liquid from a source, the primary reservoir 100, that may have a volume a multiple of volumes greater than the interstitial volume of the foam body. That is, only a liquid volume equaling a predetermined interstitial volume for a foam body 220 subject to the compression in a reagent chamber 200 is retained within the foam body. The present configuration thereby provides a known and effectively measured liquid volume within each reagent chamber 200 without requiring individual measuring or secondary procedures.

Further, as the foam body 220 may be impregnated with a variety of chemical agents such as desired reagents, buffers, surfactants, and anticoagulants, an accurate exposure of a known volume of blood to a known amount of chemical may be achieved within the foam body. Therefore, the reagent chamber 200 may

be employed to alter or treat the liquid sample retained therein with a relatively high degree of accuracy without requiring separate measurements or procedures. The reagent chambers 200 are filled from the bottom to provide the retention of a known volume upon exposure to an unmeasured larger liquid volume.

In the case where it is desired to introduce sample liquid into additional reagent chambers, the process may be repeated. Alternately, it is possible to fill several reagent chambers concurrently by rupturing each of the seals 122, 124, 126. Since each reagent chamber 200 has a unique reagent chamber pressure port, the reagent chambers may be filled simultaneously, or sequentially in any order. In all cases, the volume of liquid introduced into each reagent chamber may be accurately accomplished since each reagent chamber 200 and the corresponding foam body 220 are designed to draw and retain only a predetermined maximum volume of liquid from a source.

Therefore, the present invention permits a transfer of liquid from an unmeasured volume in the primary reservoir 100 to a plurality of downstream chambers 200, wherein each chamber will retain only a predetermined maximum volume of liquid and still define an ullage space. Thus, passive transfer is accomplished from the primary reservoir 100 to the reagent chambers 200. The passive transfer is controllable, as the self acting fluid flow is de minimus or non existent while the first and second venting ports are closed. Upon opening the first venting port, the passive fluid transfer is initiated.

For the capillary-active pressure regime, the sample liquid may now be transferred from a reagent chamber to the corresponding sensing cell.

For the active pressure regime, the liquid sample received in the reactant chambers may be subjected to an active mixing stage if desired, prior to transferring the liquid from a reagent chamber to a sensing cell. For this stage, pressurized gas is introduced to the reactant chamber to activate the bubble formation at the bubble orifice 217. Specifically, a constant flow rate of gas is presented to the bubble orifice 217. The bubble orifice 217 causes a sequence of bubbles to form and detach from the bubble orifice. Each bubble forms at the bubble orifice 217 and subsequently separates from the bubble orifice. The bubble then ascends through the liquid to the surface of the liquid.

Specifically, gas is introduced from a positive pressure source through the relevant sensing cell pressure port, for example, port 132 in the case of the reagent chamber retained in well 82. The gas travels through rupture port 138 and the control pathway 8, from sensing cell pressure port through hole 366 along the

grooved recess to the sensing cell outlet through hole 268. The gas passes through the sensing cell (which does not yet include any sample liquid) to the sensing cell inlet through hole travels along the grooved recess to reagent chamber outlet 356 connected with the relevant standpipe 16 and bubble orifice 217.

Depending upon the bubble frequency (flow rate through the bubble orifice), the particular liquid parameters and the mixing chamber construction, as few as 1 to 15 bubbles may be required to form a substantially homogeneous mixture in the reagent chamber 200, with three to six bubbles generally being sufficient.

Prior to introducing bubbles into the reagent chamber 200, the volume of liquid disposed in the chamber forms a meniscus at the surface, as shown schematically in Figure 20. The exact profile of the meniscus is determined by the characteristics of the liquid and the material of the reagent chamber 200. However, the meniscus often directs a direction of travel of a bubble reaching the surface. As the top of a bubble contacts the meniscus, the curvature of the meniscus and the buoyancy of the bubble cause the bubble to travel in a radial direction toward a periphery of the of the reagent chamber 200.

It is believed a number of mechanisms may be involved in the mixing process. The following sets forth the present understanding.

Preferably, the system is configured so that a bubble travels less than ten times its height from release at the bubble orifice to the surface. In a more preferred construction, the bubble travels less than twice its height and most preferred travels a vertical distance on the order of its own height, or less.

As a bubble forms at the bubble orifice 217, a boundary layer is formed around the bubble. The liquid that forms the bubble boundary layer is substantially taken from the adjacent the bottom boundary layer. Thus, liquid from adjacent the bottom boundary layer surrounds the bubble. As the bubble expands prior to separation from the bubble orifice more liquid is drawn from adjacent the bottom boundary layer and the lower part of the mixing chamber.

The bubble then separates from the bubble orifice 217 and ascends to the meniscus. The departing or separation of the bubble from the bubble orifice 217 causes an impulse resulting in a localized plume above the bubble. As the bubble is generally surrounded by material from the bottom wall boundary layer, the plume functions to transfer liquid upward from the bottom of the mixing chamber. Some bubbles do not immediately penetrate the surface of the liquid and are

directed by the curvature of the meniscus toward the side wall. The bubble may then rupture, thereby releasing the bubble boundary layer near or at the surface of the liquid. Thus, liquid from adjacent the bottom boundary layer is transported to the surface or upper portion of the liquid in the reagent chamber 200.

Thus, it appears that rather than creating a continual upwelling from the ascendance of a series of bubbles, the mass transfer by the individual bubbles is a dominant mixing mechanism.

It has been observed that as the bubble is formed at the bubble orifice 217, the vertical position of the intersection of the meniscus and the mixing chamber rises. The formation of a bubble at the bubble orifice 217 causes the entire surface level of the liquid to climb up along the side wall. The meniscus rises along the face of the side wall as the bubble forms, and then descends along the side wall as the bubble collapses.

It has been further observed that initial bubbles in the mixing process may cluster or form a single layer at the surface of the liquid. Then, the collected layer of bubbles at the surface substantially simultaneously rupture and thereby induce significant mixing.

It is also believed the energy released upon rupture of a bubble at the surface contributes to the mixing by sending sufficient energy through the liquid to agitate the boundary layers.

Further, the ascension of a bubble through the liquid creates a upward current along the longitudinal axis. This upwelling is balanced by a downwelling along the side wall to the bottom wall. Thus, a current may be generated in the reagent chamber 200. However, at sufficiently low gas flow rates through the bubble orifice, a current may not be formed as the friction effects of the water dampen the flow and prevent a sustained upwelling along the path of bubble ascent.

Depending upon the application of the system, excessive bubble retention at the surface or foaming may be undesirable. It has been found that surfactants assist in reducing the tendency for foam to persist. In addition, it has been found that at relatively low flow rates through the bubble orifice (approximately 10 to 15 μL of gas per second) some foaming is common. However, at gas flow rates through the bubble orifice of 35 to 50 μL per second, foaming has been observed to be reduced. In addition, the higher flow rates of gas may tend to establish stronger currents in the mixing chamber and reduce the time to form a homogeneous mixture.

In addition, as a bubble rises through the liquid, counter rotating eddies are formed behind each bubble. These eddies loosely resemble the turbulent vortices in conventional mixing regimes.

Accordingly, the present invention allows for reproducible and repeatable mixing or agitation between different samples. In addition, operator exposure to the liquid during agitation or mixing is significantly reduced.

In certain circumstances, it is necessary to continue agitation/mixing of a liquid sample beyond the point at which reagents have been dissolved. For example, there are instances where it is necessary to for cells in a liquid sample to come into contact with insoluble reagent particle surfaces; other cell surfaces; to some other surface with which the cells will react, or interact. In such cases, prolonged agitation or mixing may be accomplished by continued bubbling to expose cells to ensure that all of the cells have had an adequate opportunity to contact such other reaction surfaces in the liquid sample.

Upon the necessary mixing or residence time in the reagent chambers 200, the treated liquid is transferred to the sensing cells 300.

In both the capillary-active pressure regime and the active pressure regime, liquid is transferred to the corresponding sensing cell 300 in a generally similar manner. The sensing cell pressure ports 132, 134, 136 are connected to a negative pressure source, and a negative pressure is applied to the corresponding sensing cell 300 and hence reagent chamber 200. The corresponding reagent chamber pressure port 122, 124, 126 is vented so that liquid is drawn into the transfer manifold through the outlet in the reagent chamber while the venting port 44 is sealed.

Alternatively, or in addition, in the capillary-active regime a slight positive pressure may be applied to the reagent chamber pressure port 122, 124, 126. The positive pressure from the reagent chamber pressure port acts on the top of the foam body. This positive pressure causes fluid above the projecting stem 351, located within the foam body, to pass into the outlet hole at the top of the projecting stem and into the transfer manifold. As the projecting stem is preferably located within the foam body 220, a blood volume remains in the reagent chamber 200 below the outlet hole of the projecting stem. This lower volume of liquid acts a barrier and substantially precludes liquid from the well becoming entrained in the outgoing flow. This barrier function is helpful as the liquid in the peripheral well is likely to have a different chemical concentrations than that liquid that has been fully exposed to the foam body.

The respective volume of the primary reservoir 100, the reagent chambers 200, the distribution manifold 150, the transfer manifold 250 and the sensing cells 300 are preferably sized to retain a volume of liquid in the foam member 220 above the projecting stem upon operably filling the sensing cell 300. Thus, relatively uniform exposure of the liquid in the reagent chamber 200 to the particular chemical is achieved. That is, the liquid that is transferred from the reagent chamber 200 is taken from within the foam body 220 and has a reduced time exposed to air. The positive pressure on the first venting port causes liquid to flow through the outlet hole into the transfer manifold and into the sensing cell. That is, the liquid from a reagent chamber passes through the outlet and an associated transfer channel to enter a unique sensing cell. This is an active transfer of liquid.

In both regimes, as the liquid flows into the corresponding sensing cell 300, the flow resistors in the sensing cell may be used to assist in the even filling of a plurality of sensing cells. Thus, as a particular sensing cell 300 is filled, the resistance to continued filling increases and the flow is then favored to a sensing cell that exhibits less resistance. The flow preference change does cause flow to divert from its travel to a given sensing cell to another sensing cell, but rather the pressure acting on the reagent chambers exerts greater flow in a transfer channel exhibiting a lesser resistance to flow. This aids in ensuring that each of the sensing cells is adequately filled from the associated reagent chamber without requiring separate confirmation or procedure. Alternatively, the sensing cell pressure ports may be controlled to ensure a slight overfill of the sensing cell. The vent path from the sensing cell outlet to the sensing cell pressure port is of sufficient volume to retain the overfill liquid. Preferably, the vent path has a sufficient volume such that upon voiding the reagent chamber and the sensing cell being filled, liquid will not pass through the sensing cell pressure port and from the cartridge. Alternatively, as the air volume within the control pathway 8 and the liquid pathway 6 are selected so that the air acts as incompressible, the volume of liquid transferred to a sensing cell may be controlled by admitting venting air via the pressure port.

As the sensing cells 300 are located beneath the evanescent waveguide, gravity induced separation or particulate sedimentation within the sensing cell will occur away from the evanescent waveguide, and a layer of "relatively clear" liquid will be located adjacent and in contact with the waveguide. A stratum of reduced sedimentation will be located nearer the waveguide than a stratum of

greater sedimentation. Alternatively, the cartridge is constructed to locate the liquid above the waveguide 70 and negatively buoyant particles tend to settle towards the waveguide.

Thus, the present cartridge 10 may include a plurality of sensing cells 300 and associated reagent chambers 200 such as three sensing cell with each sensing cell cooperatively associated with an individual reagent chamber. As reverse flow or upstream flow is precluded, liquid treated in a respective reagent chamber is not exposed to or contaminated by a liquid in a different reagent chamber. Thus, each sensing cell may be filled with a uniquely treated volume of the liquid, that has not been contaminated with the chemical process associated with any other reagent chamber or sensing cell. It has been found that for typical flow rates of approximately 15 to 30 μL per second within the liquid flow path, that liquid transfer from the primary reservoir 100 to sequentially fill three reagent chambers and subsequently fill three sensing cells may occur in less than one minute.

While the invention has been described with reference to preferred embodiments, it will be understood by those skilled in the art that various changes may be made and equivalents may be substituted for elements thereof without departing from the scope of the invention. In addition, many modifications may be made to adapt a particular situation of material to the teachings of the invention without departing from the scope of the invention. Therefore, it is intended that the invention not be limited to the particular embodiments disclosed as the best mode contemplated for carrying out this invention, but that the invention will include all embodiments falling within the scope and spirit of the appended claims.

We Claim:

1. A device for transferring a liquid to a sensing cell, comprising:
(a) a primary reservoir having an inlet port;
(b) a reagent chamber fluidly connected to the primary reservoir;
(c) a first venting port fluidly connected to the reagent chamber;
(d) a sensing cell fluidly connected to the reagent chamber; and
(e) an optical waveguide optically connected to the sensing cell; and
(f) a second venting port fluidly connected to the sensing cell.

2. The device of Claim 1, comprising a plurality of reagent chambers fluidly connected to the primary reservoir.

3. The device of Claim 2, wherein the plurality of reagent chambers are fluidly connected to the primary reservoir via a distribution manifold.

4. The device of Claim 2, comprising a plurality of sensing cells, each sensing cell independently fluidly connected to a respective reagent chamber.

5. A cartridge for receiving and distributing a liquid sample, comprising:
(a) a primary reservoir having a single inlet and a single outlet;
(b) a plurality of reagent chambers, each reagent chamber having an inlet;
(c) a distribution manifold fluidly connecting the primary reservoir outlet to each regent chamber inlet;
(d) a first plurality of venting ports, each of the first plurality uniquely connected to a corresponding reagent chamber;
(e) a plurality of sensing cells, each sensing cell having an inlet and an outlet;
(f) a plurality of transfer channels uniquely connecting a reagent chamber to a sensing cell; and
(g) a second plurality of venting ports, each of the second plurality of venting ports uniquely connected to a corresponding sensing cell.

6. The cartridge of Claim 5, further comprising an optical waveguide adjacent the plurality of sensing cells.

7. The cartridge of Claim 5, further comprising a rupturable seal occluding the first and the second plurality of venting ports.

8. The cartridge of Claim 5, further comprising a cartridge door moveable between an open position exposing the primary reservoir inlet and a closed position substantially occluding the primary reservoir inlet.

9. A cartridge for receiving and distributing a liquid sample, comprising:

(a) a liquid pathway extending from a primary reservoir to a downstream reagent chamber, the liquid pathway extending from the reagent chamber to a downstream sensing cell;

(b) a first vent path and a second vent path connected to the liquid pathway to locate the reagent chamber and the sensing cell intermediate the first vent path and the second vent path.

10. The cartridge of Claim 9, further comprising an optical waveguide optically connected to the sensing cell.

11. The cartridge of Claim 9, further comprising a plurality of reagent chambers and a distribution manifold in the liquid pathway intermediate the primary reservoir and the reagent chambers.

12. The cartridge of Claim 9, wherein, the primary reservoir includes a single inlet port.

13. A cartridge for presenting a sample, comprising:

(a) a primary reservoir having a single inlet port for receiving a sample of whole blood;

(b) a reagent chamber downstream of the primary reservoir having a fill port, an outlet port and a spaced apart venting port, the fill port located in a bottom portion of the reagent chamber and fluidly connected to the primary reservoir;

(c) an evanescent waveguide;

(d) a sensing cell optically connected to the evanescent waveguide and fluidly connected to the reagent chamber; and

(e) a second venting port fluidly connected to the sensing cell.

14. A cartridge, comprising:

(a) an evanescent waveguide; and

(b) a sensing cell partially defined by a portion of the evanescent waveguide and a portion of the cartridge, wherein at least a section of the portion of the cartridge is black.

15. The cartridge of Claim 14, wherein the portion of the cartridge is a gasket.

16. The cartridge of Claim 15, wherein the gasket is a laminate including a black layer.

17. An apparatus for exposing an evanescent wave emanating from a sensing surface to a liquid sample, comprising:

(a) a sensing cell selected to dispose a stratum of the liquid in contact with the sensing surface, the stratum having a sediment concentration less than a second stratum spaced from the sensing surface.

18. An apparatus for exposing an evanescent wave emanating from a sensing surface to a liquid sample, comprising:

(a) a sensing cell selected to form a first stratum of the liquid in contact with the sensing surface and a second stratum, wherein the first stratum is intermediate the second stratum and the sensing surface, and the first stratum has a sediment concentration less than the second stratum.

19. A device for drawing a measured volume of a liquid from a source having a liquid volume greater than the measured volume, comprising:

(a) a primary reservoir having a liquid volume greater than the measured volume;

(b) a ventable first reagent chamber;

(c) a first fluid connection between the first reagent chamber and the primary reservoir, the first fluid connection having a sufficiently small volume to render air in the first fluid connection substantially incompressible;

(d) a first sensing cell fluidly connected to the reagent chamber to define a transfer manifold having a sufficiently small volume to render air in the transfer manifold as substantially incompressible.

20. The device of Claim 19, further comprising an optical waveguide optically coupled to the first sensing cell.

21. A device for transferring a liquid to a sensing cell, comprising:

(a) a primary reservoir having an inlet port;

(b) a reagent chamber having a well fluidly connected to the primary reservoir;

(c) a foam member disposed in the reagent chamber to expose a portion of the foam member to the well;

(d) a venting port fluidly connected to the reagent chamber;

(e) a sensing cell fluidly connected to the reagent chamber; and

(f) a second venting port fluidly connected to the sensing cell.

22. The apparatus of Claim 21, wherein the well extends about a periphery of the reagent chamber and is located in a bottom portion of the reagent chamber.

23. The device of Claim 21, wherein the fluid connection of the primary reservoir and the reagent chamber is sized to create a pressure head in the primary reservoir and a flow force from a surface tension of the liquid sufficient to initiate flow upon opening the venting port.

24. The device of Claim 21, further comprising a plurality of reagent chambers fluidly connected to the primary reservoir and a corresponding plurality of sensing cells fluidly connected to corresponding reagent chambers.

25. The device of Claim 21, further comprising a cover for selectively closing the primary reservoir.

26. The device of Claim 21, wherein the foam member is an open cell foam.

27. The device of Claim 21, wherein the primary reservoir includes a single inlet port.

28. The device of Claim 21, wherein spaced apart portions of the foam member are subject to different compressive forces.

29. The apparatus of Claim 21, wherein one of the reagent chamber and the foam member is a conic section.

30. The device of Claim 21, further comprising an evanescent waveguide optically coupled to a sensing cell.

31. The device of Claim 21, further comprising an outlet port located within the foam member.

32. The device of Claim 21, wherein the reagent chamber and the sensing cell are sized such that upon operably filling the sensing cell, liquid remains in the reagent chamber below the outlet port and above the outlet port.

33. The device of Claim 21, wherein the sensing cell includes a back pressure generator for creating a given back pressure upon a predetermined volume of liquid entering the sensing cell.

34. The device of Claim 21, wherein the second venting port is selected to accommodate a liquid overfill of the sensing cell.

35. The device of Claim 21, further comprising a manifold fluidly connecting the primary reservoir and the reagent chamber, the manifold sized to provide a flow inducing force resulting from a surface tension of the liquid.

36. A device for drawing a first predetermined maximum volume of liquid from a source having a liquid volume greater than the predetermined maximum volume, comprising:

(a) a reservoir having a liquid volume greater than the predetermined maximum volume;

(b) a ventable first chamber;

(c) a first fluid connection between the first chamber and the reservoir;

(d) a first foam body sized to be retained in the first chamber to form a first ullage space, the first foam body having a first interstitial volume substantially equal to the first predetermined maximum volume;

the reservoir, the first chamber, the first foam body and the first fluid connection selected to substantially preclude a liquid flow into a vented first chamber upon the first interstitial volume being filled with liquid from the reservoir.

37. The device of Claim 36, wherein the first chamber includes a well, and the well and the first foam body are selected to create a fluid flow into the

first foam body that is substantially perpendicular to a lower periphery of the foam body.

38. The device of Claim 37, further comprising an outlet port located within the first foam body.

39. The device of Claim 38, wherein the outlet port is located to remove liquid retained within the foam body prior to withdrawing liquid from the well portion.

40. The device of Claim 36, wherein a largest compressive force on the foam member is adjacent the well.

41. The device of Claim 36, wherein spaced portions of the foam body are subject to different compressive forces.

42. The device of Claim 36, wherein the foam body generally converges toward the outlet.

43. The device of Claim 36, wherein the well and the foam body are selected to expose a lower periphery of the foam body and a portion of a bottom of the foam body to the well.

44. A method of transferring liquid in a cartridge having a primary reservoir, a reagent chamber and a downstream sensing cell, comprising:

(a) opening a flow path upstream of the primary reservoir;

(b) venting the reagent chamber through a first venting port; fluidly connected to the reagent chamber;

(c) sealing a flow path through a second venting port fluidly connected to the sensing cell; and;

(d) exposing the reagent chamber to a pressure differential to induce a flow of liquid from the primary reservoir to the reagent chamber.

45. A method of transferring liquid within a cartridge, comprising:

(a) venting a regent chamber to facilitate flow from an upstream primary reservoir into the regent chamber;

(b) sealing a flow path volume upstream of the regent chamber;

(c) venting a downstream sensing cell fluidly connected to the regent chamber; and

(d) applying a pressure differential to the regent chamber to induce flow from the reagent chamber to the downstream sensing cell.

46. The method of Claim 45, further comprising sizing the chamber and the upstream reservoir to provide a pressure head in the upstream reservoir and a capillary flow to induce flow from the primary reservoir to the chamber.

47. The method of Claim 45, further comprising applying a negative pressure differential to the upstream primary reservoir to facilitate flow to the reagent chamber.

48. The method of Claim 45, further comprising locating the downstream cell adjacent an evanescent waveguide.

49. The method of Claim 45, further comprising disposing a plurality of reagent chambers downstream of the primary reservoir.

50. The method of Claim 45, further comprising disposing a back pressure generator in the sensing cell to create a flow restricting pressure upon a predetermined volume of liquid entering the sensing cell.

51. A method of forming a device for presenting a liquid sample to a sensing cell, comprising:

(a) forming a first reagent chamber;

(b) forming a primary reservoir;

(c) fluidly connecting the primary reservoir and the first reagent chamber;

(d) connecting the first reagent chamber to a first venting port;

(e) connecting the first reagent chamber to a corresponding first sensing cell; and

(f) connecting the first sensing cell to a second venting port.

52. The method of Claim 51, further comprising forming the primary reservoir to have a single inlet.

53. The method of Claim 51, further comprising operably aligning the first sensing cell with an evanescent waveguide.

54. The method of Claim 51, further comprising disposing the first reagent chamber, the primary reservoir and the first sensing cell in a cartridge.

55. The method of Claim 51, further comprising sizing the first reagent chamber and the primary reservoir to render any air in the first reagent chamber and the primary reservoir substantially incompressible.

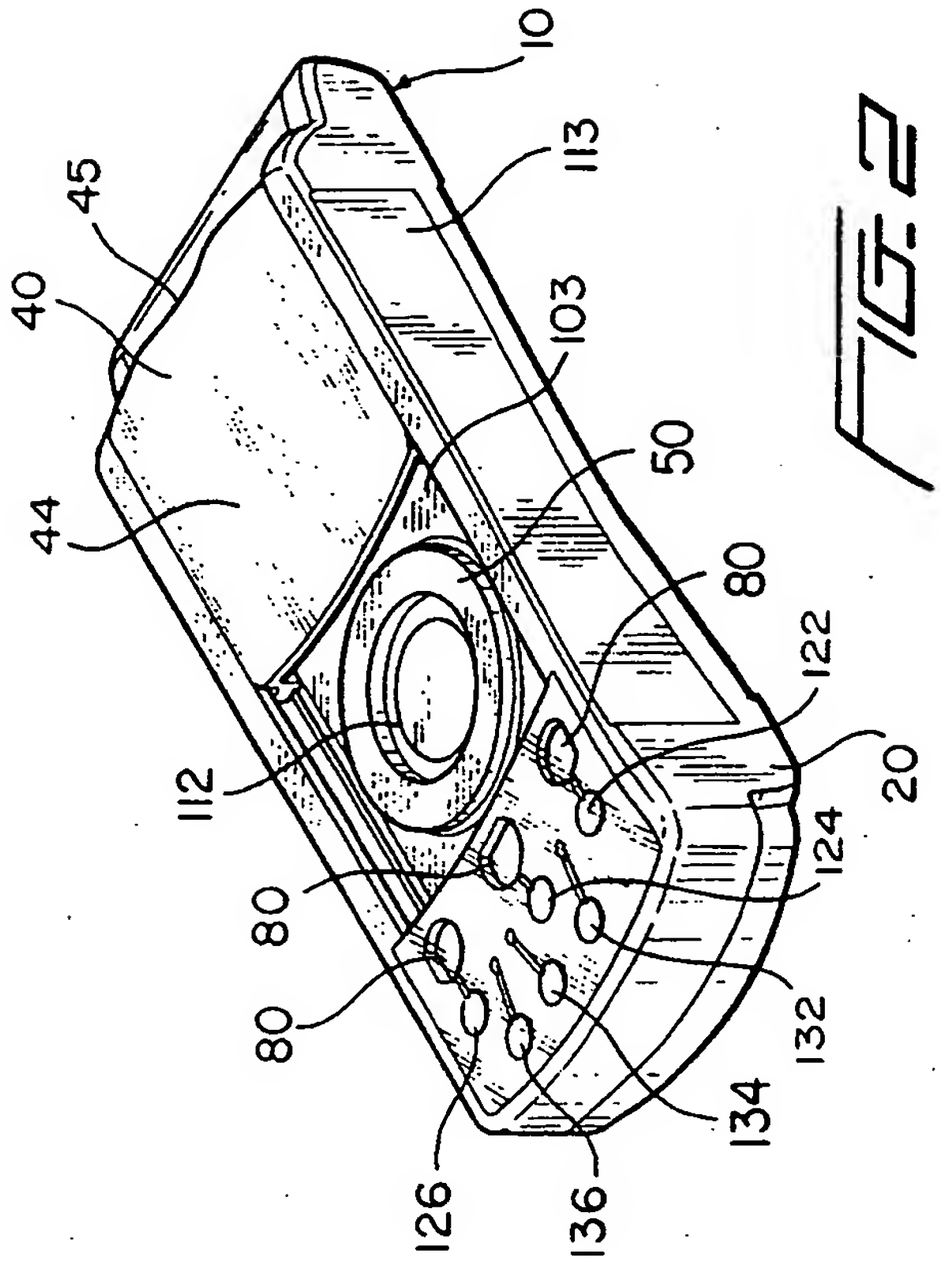
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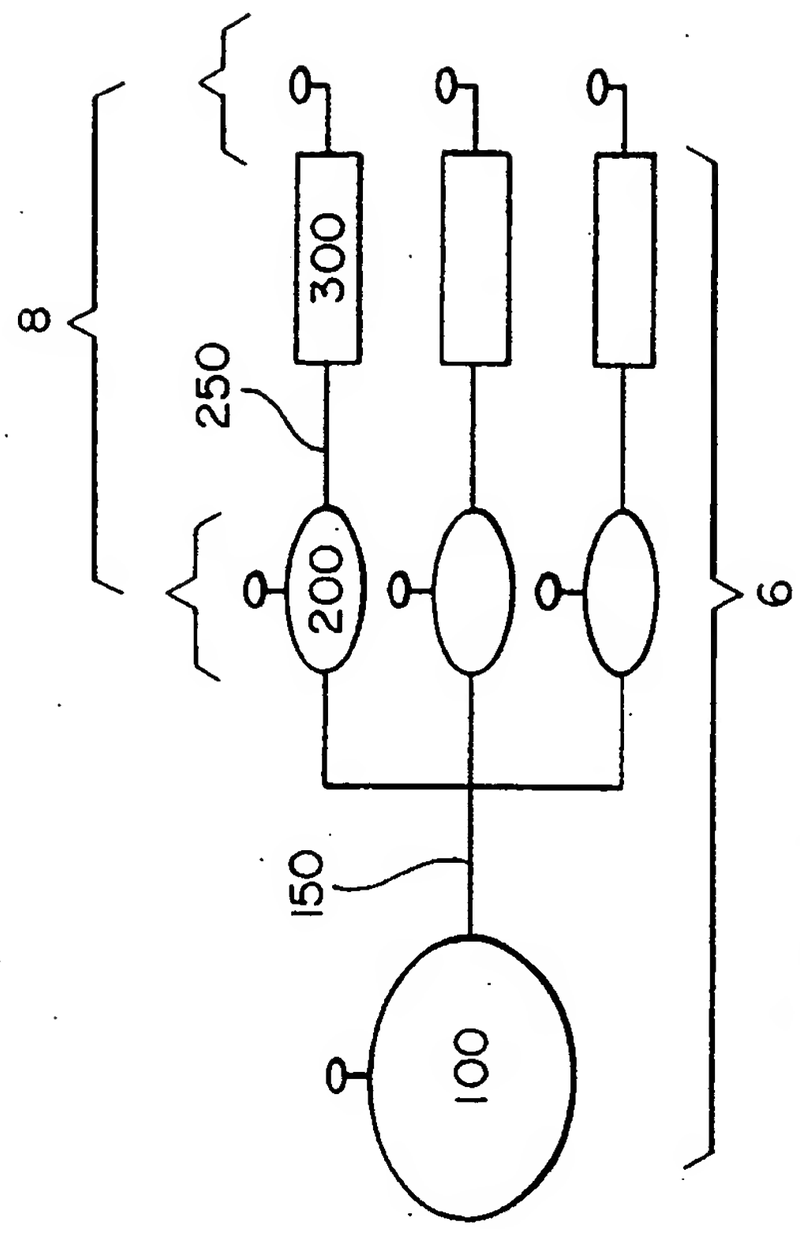
FIG. 2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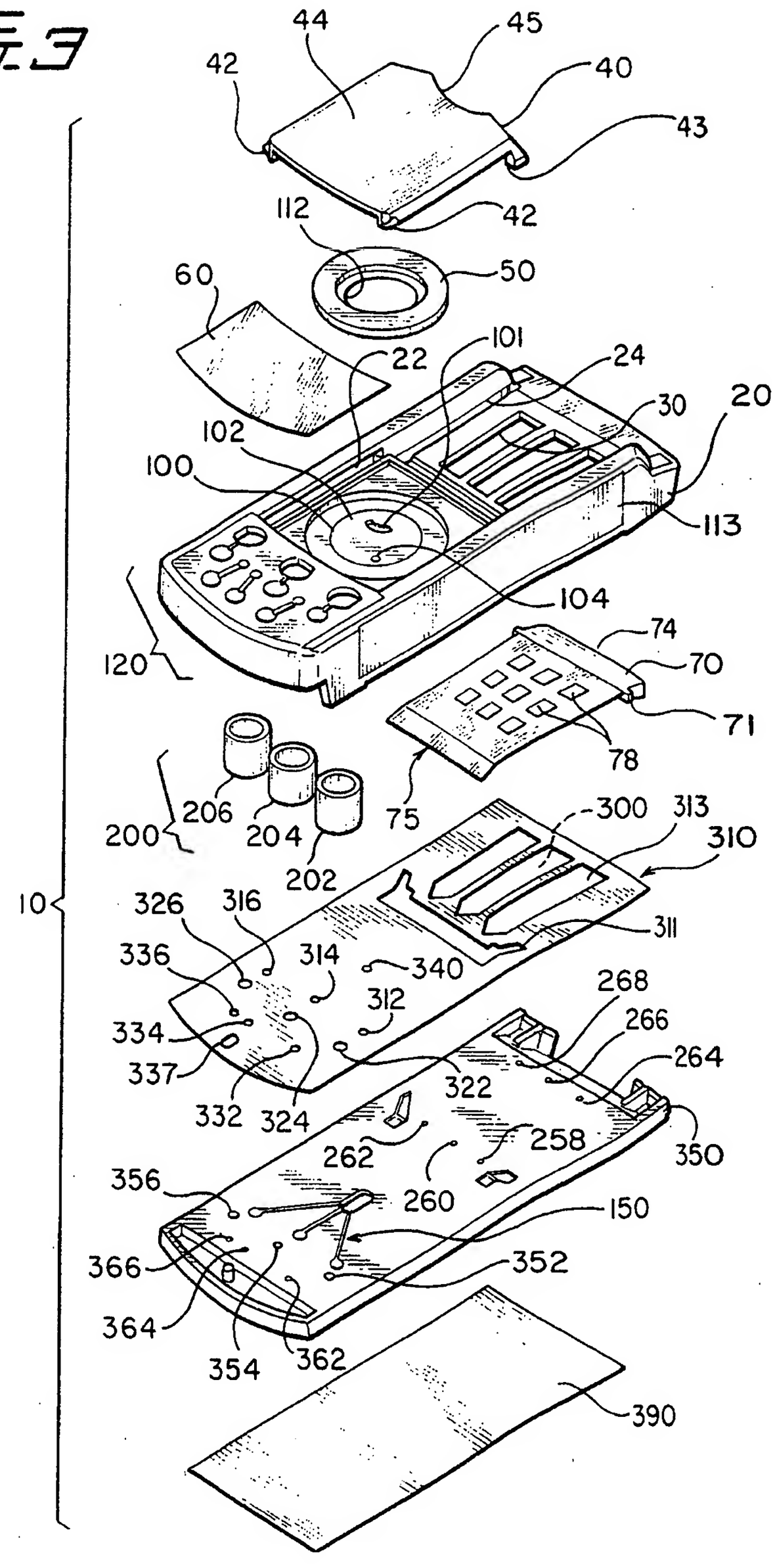
100

FIG. 1

FIG. 3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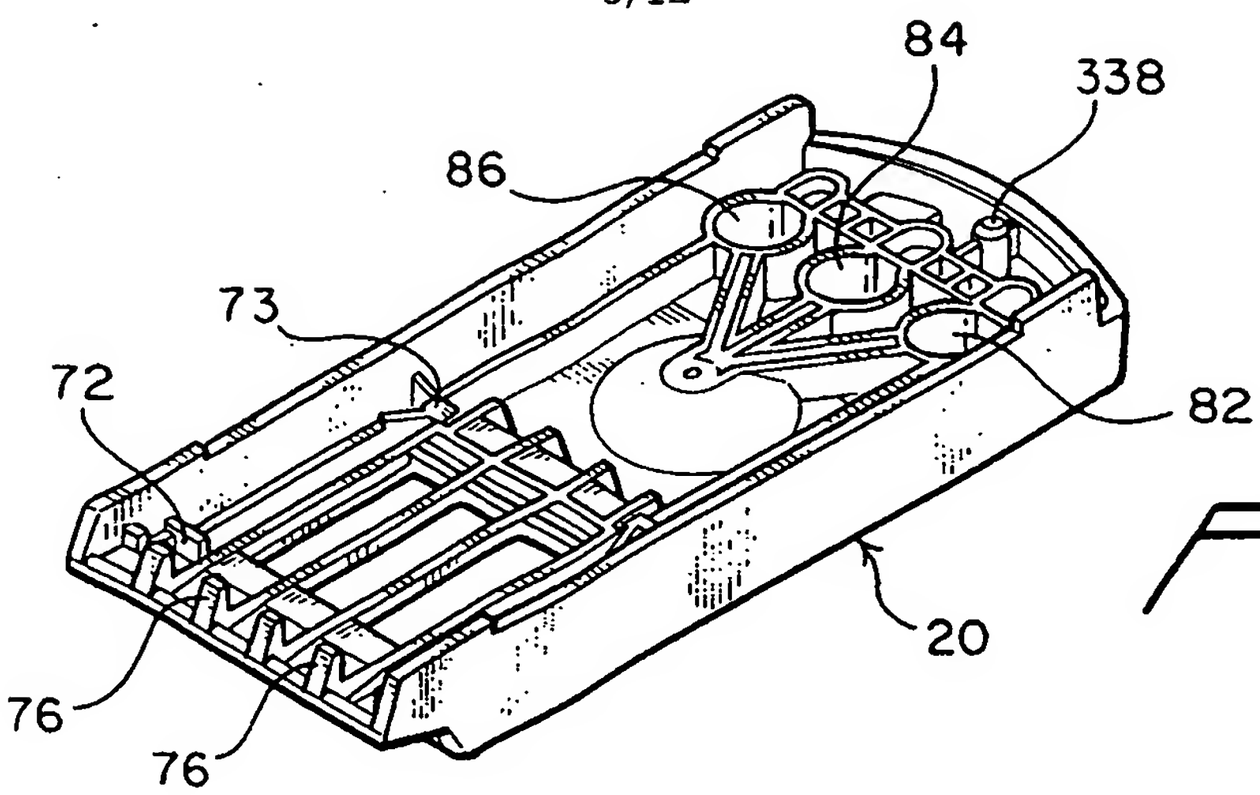
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FIG. 4

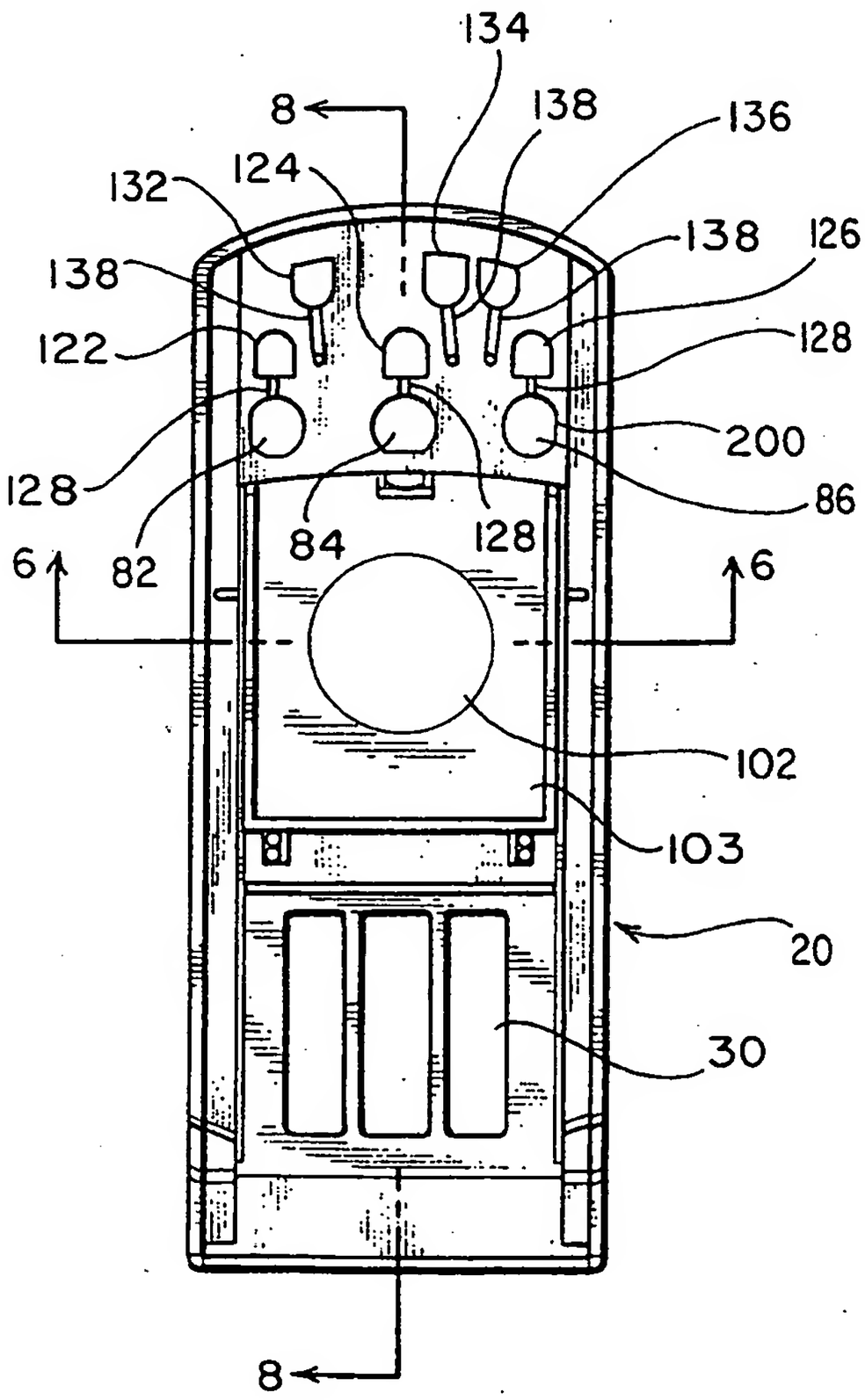
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FIG. 5

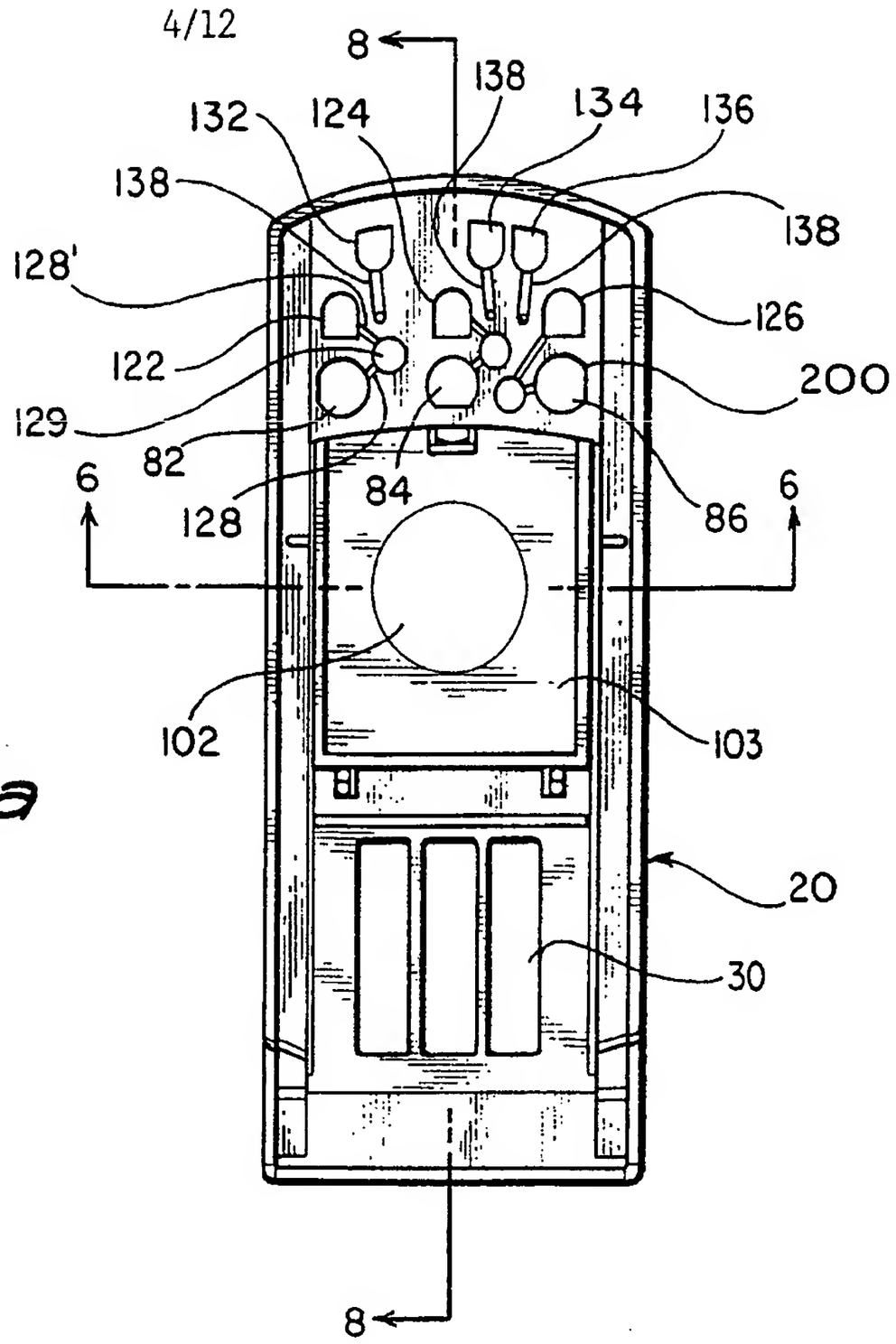
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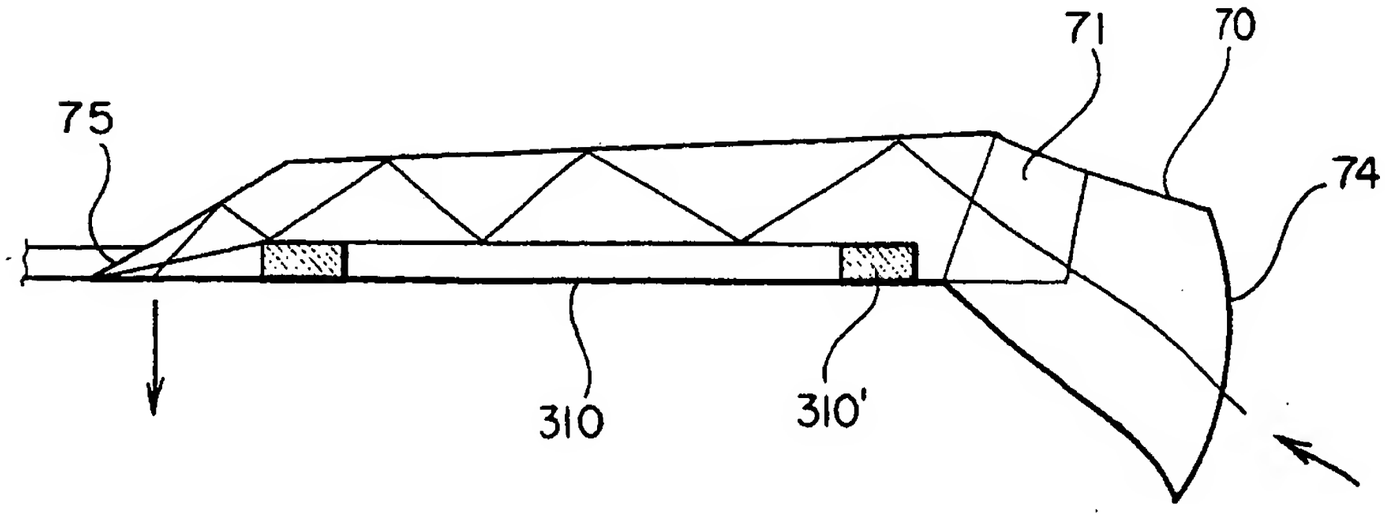
FIG. 5a

FIG. 12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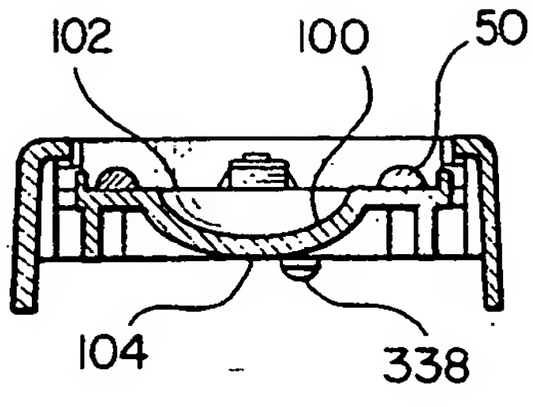

FIG. 6

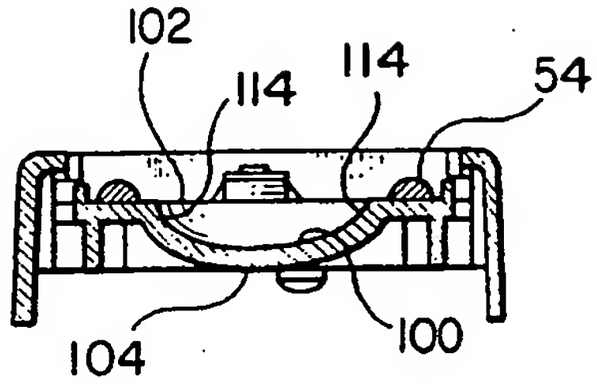

FIG. 7

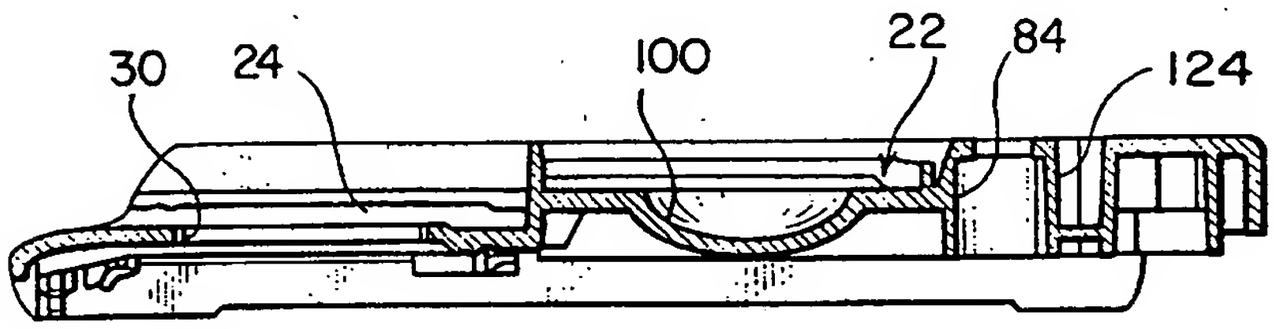

FIG. 8

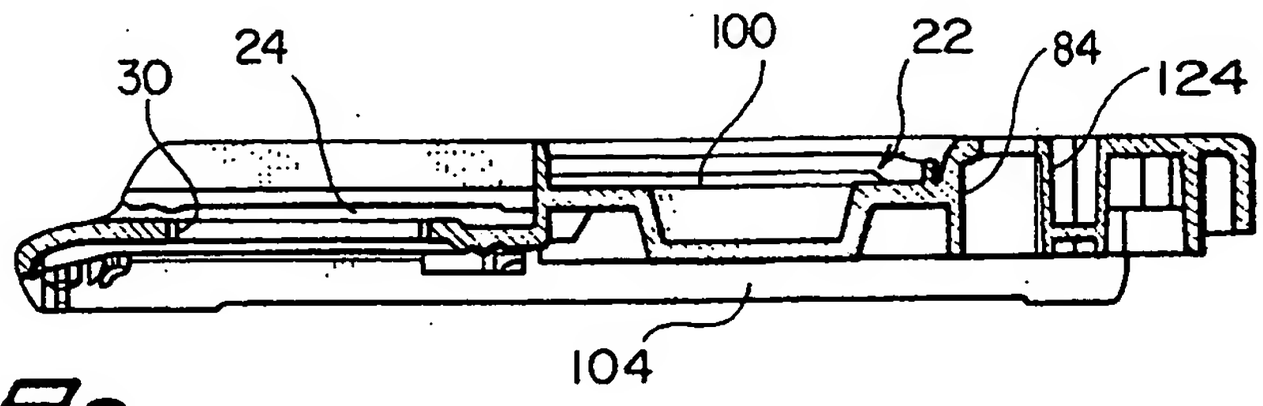

FIG. 8a

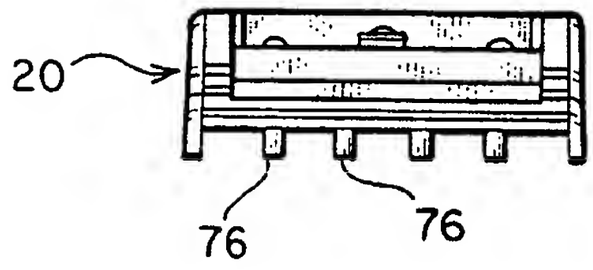

FIG. 9

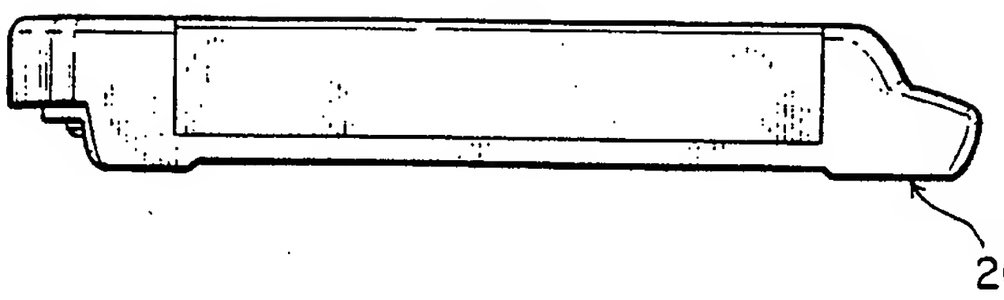

FIG. 10

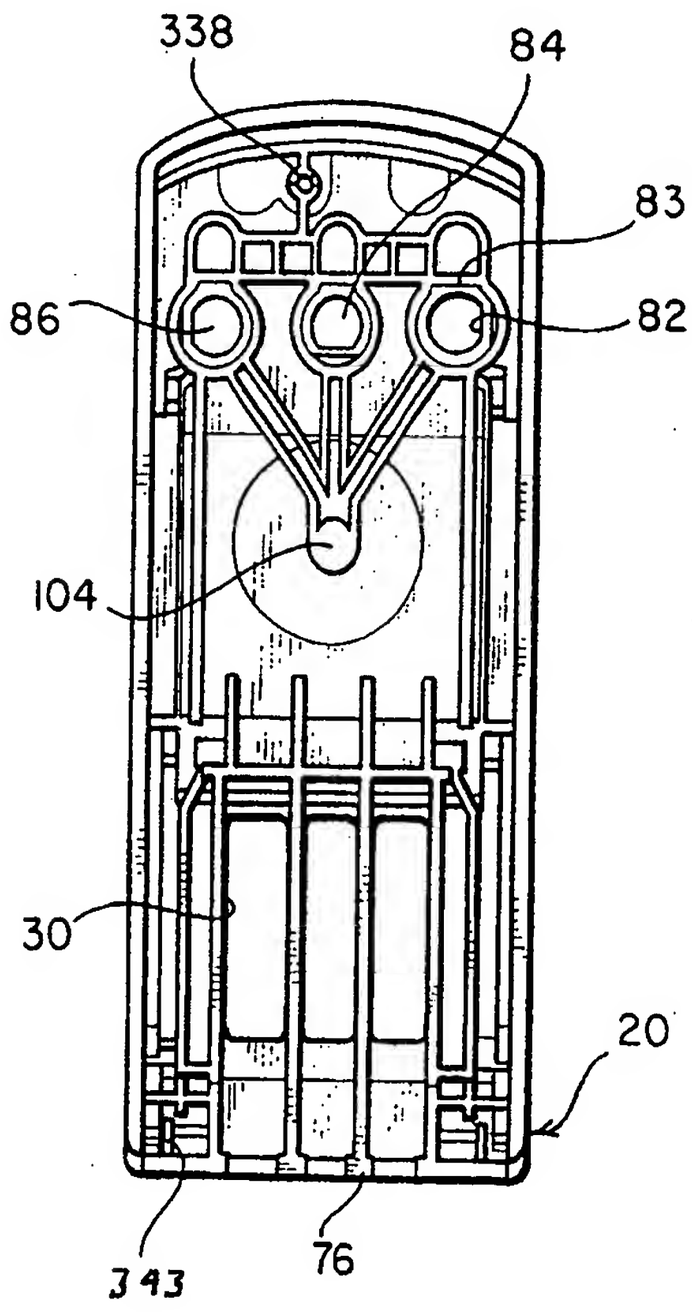

FIG. 11

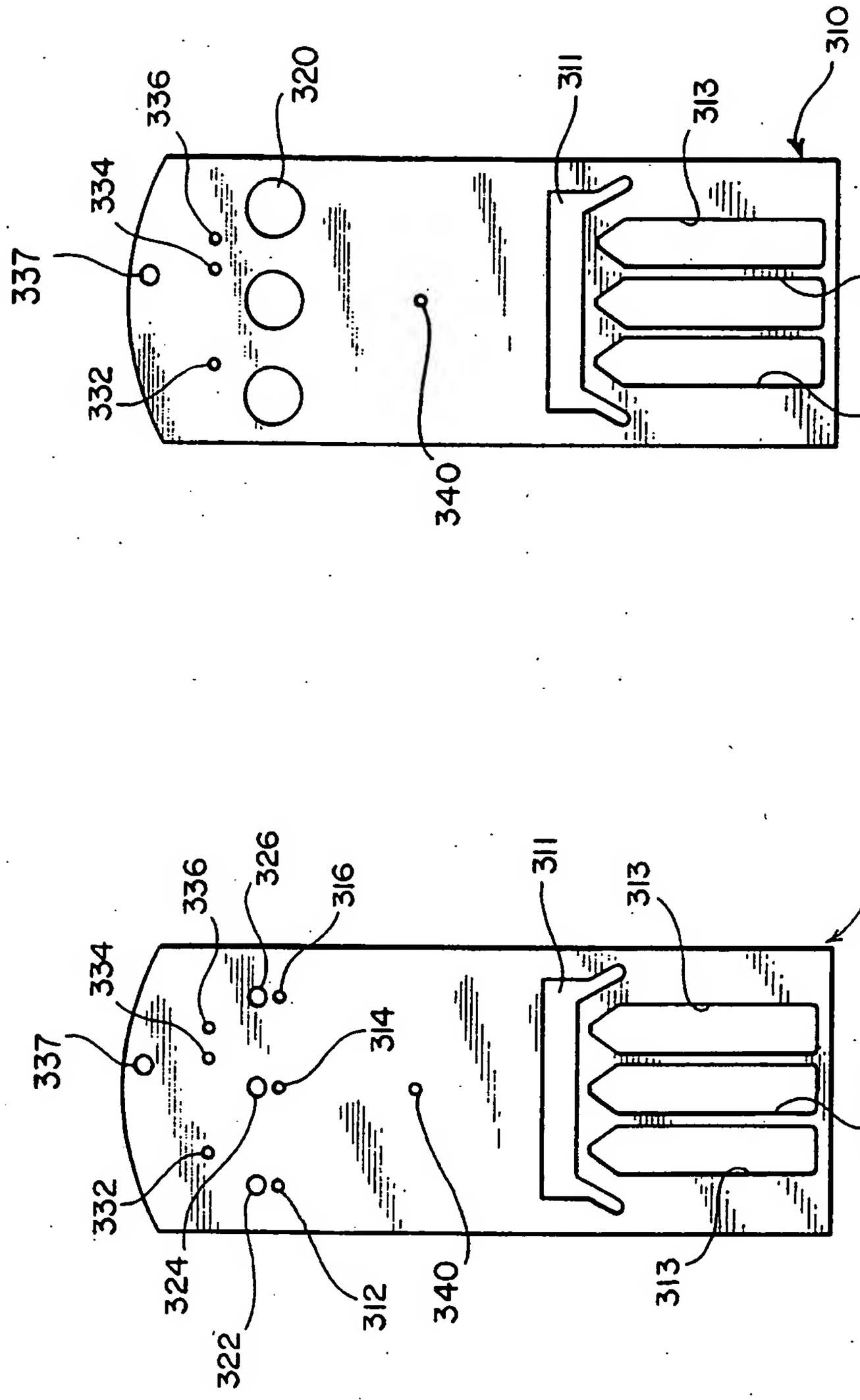
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FIG. 17

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FIG. 18

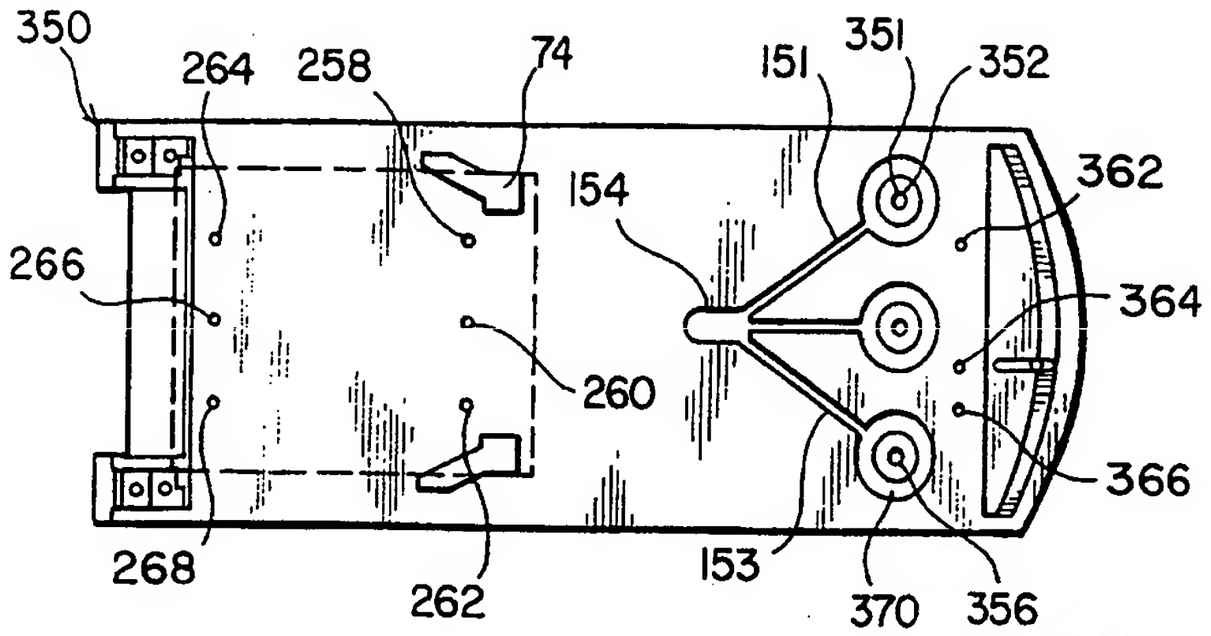

FIG. 13

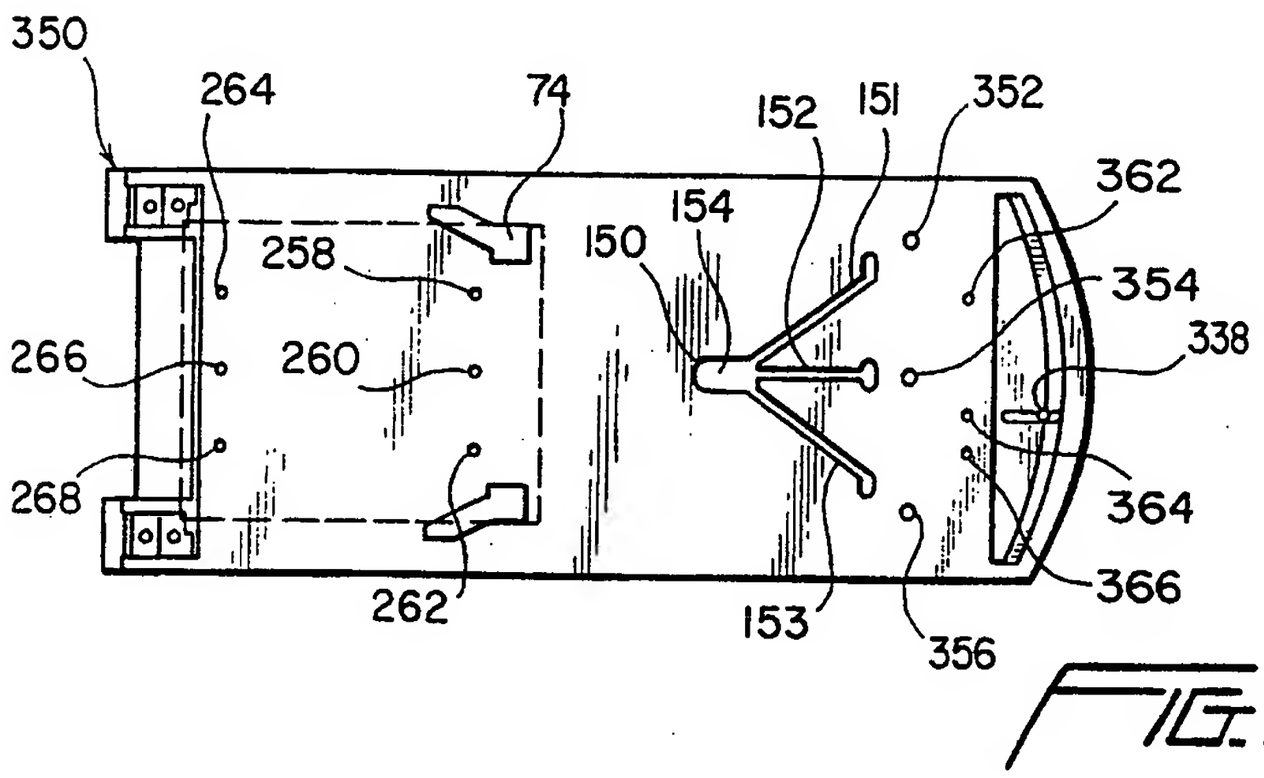

FIG. 14

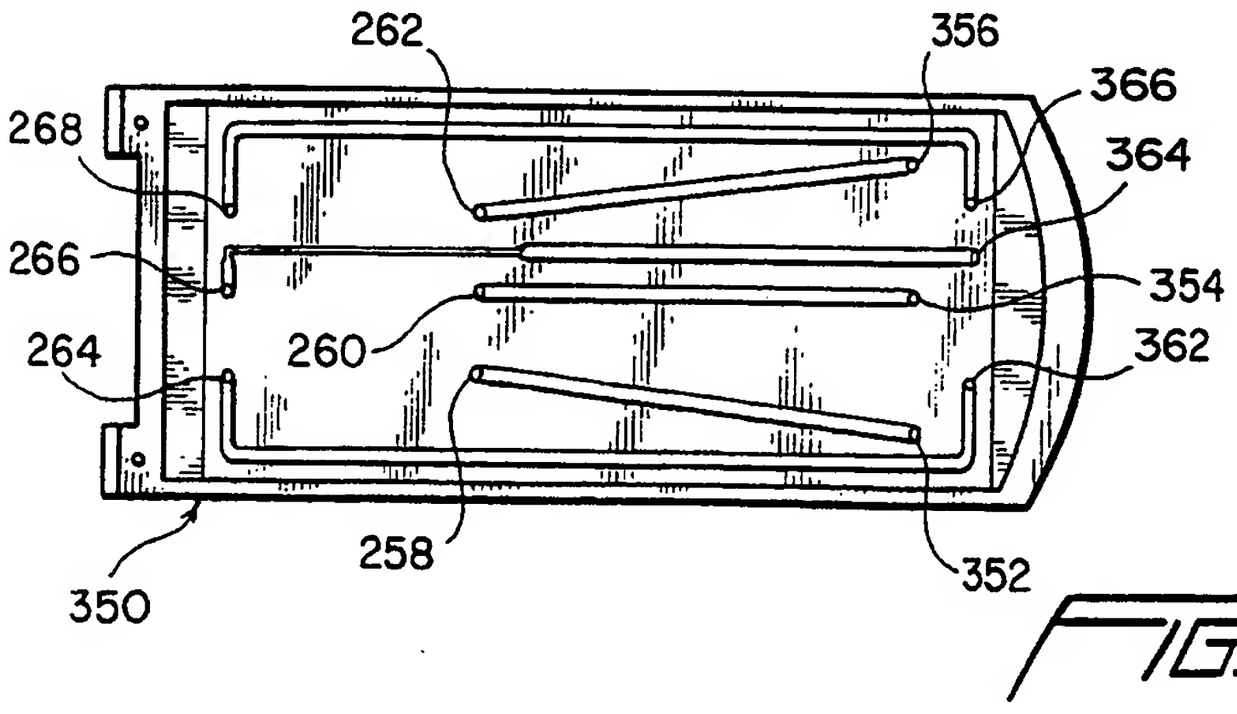

FIG. 15

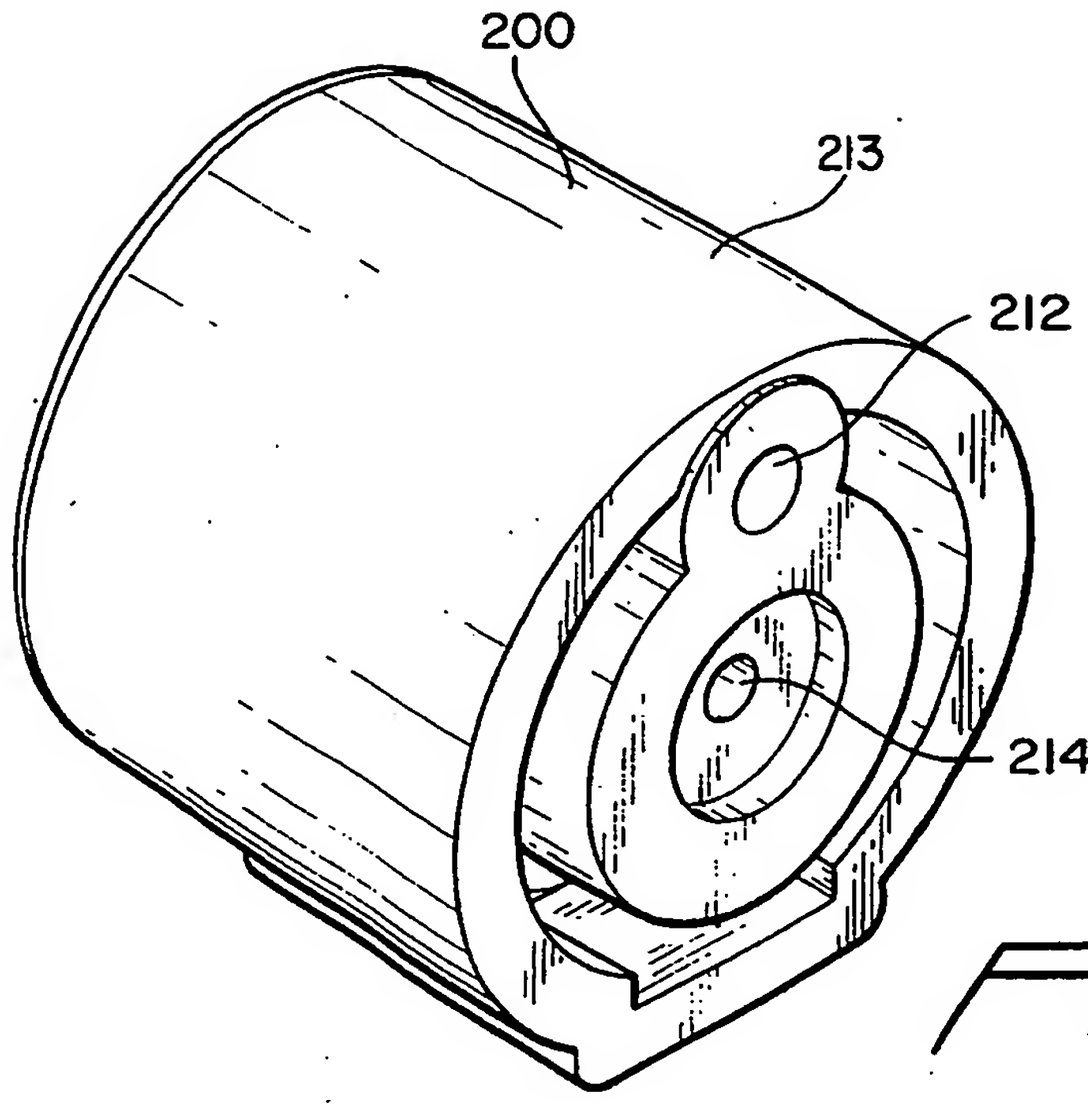

FIG. 18

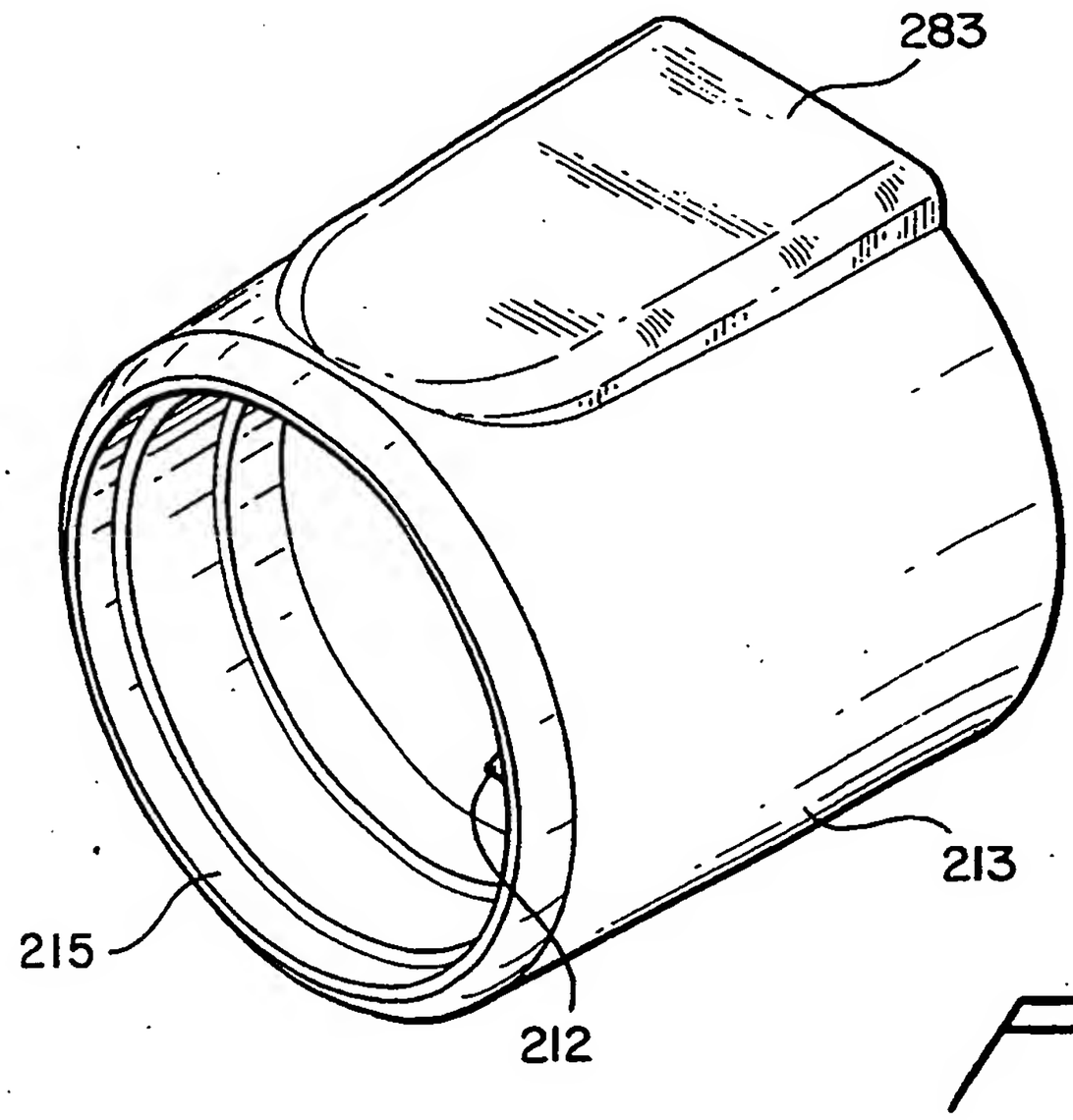

FIG. 19

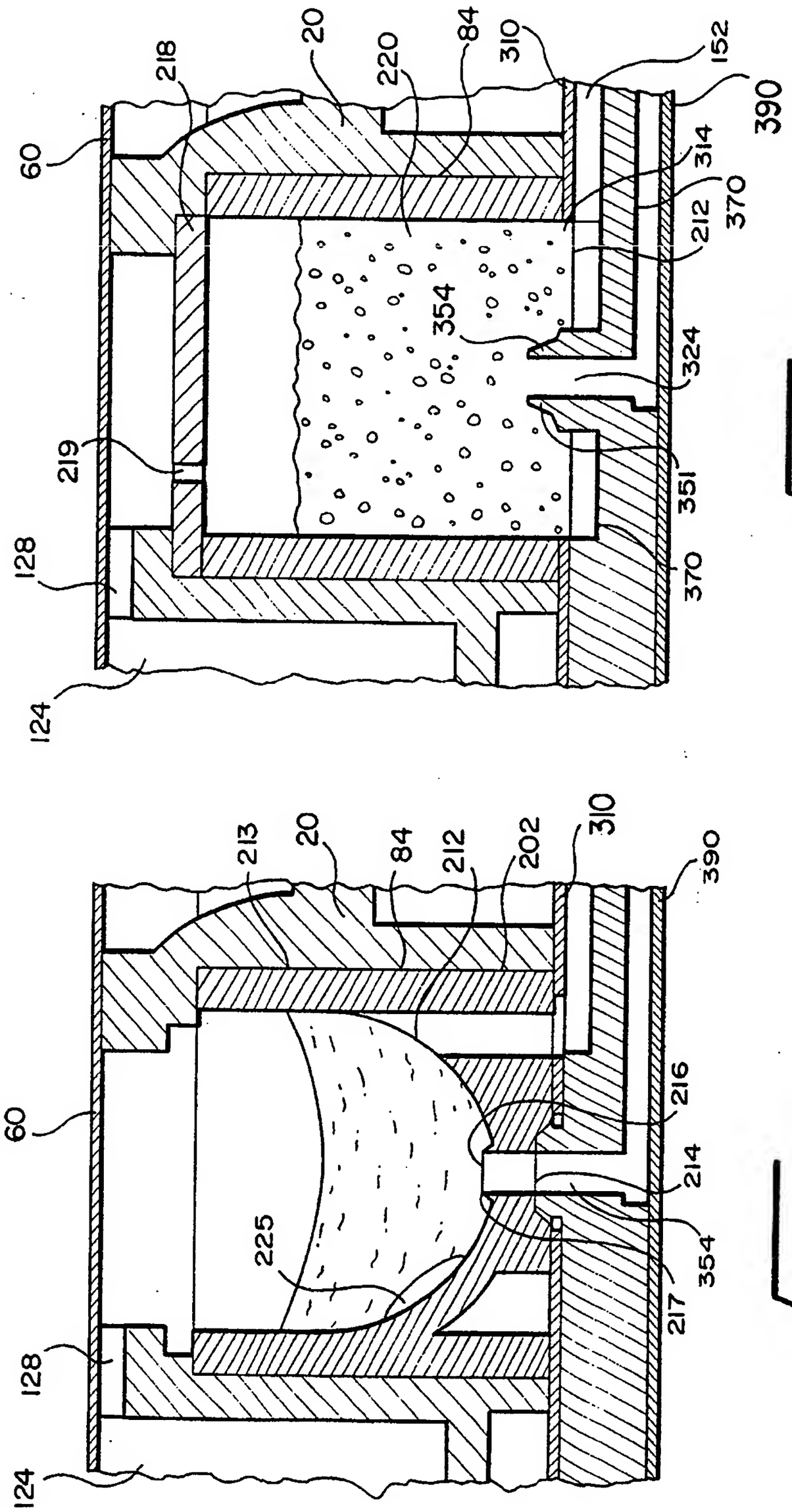
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FIG. 26

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FIG. 20

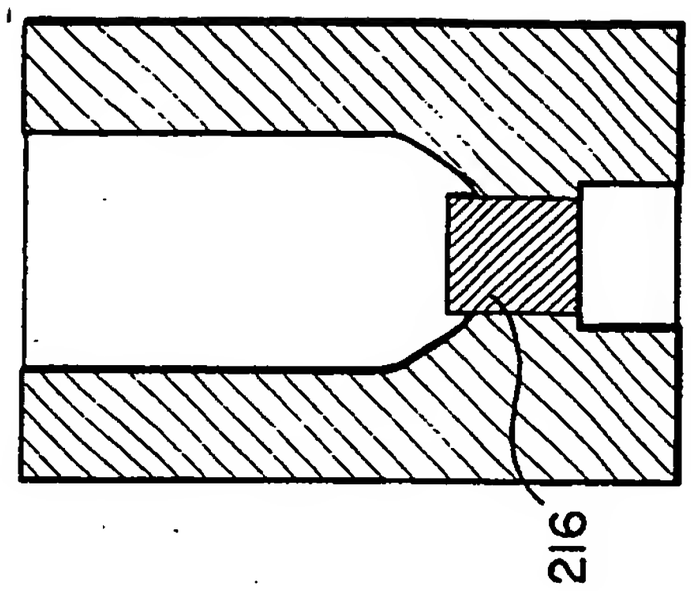

FIG. 31

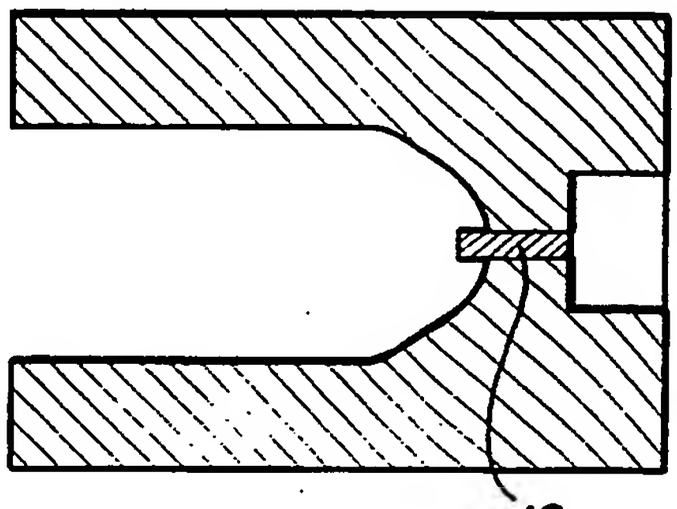

FIG. 30

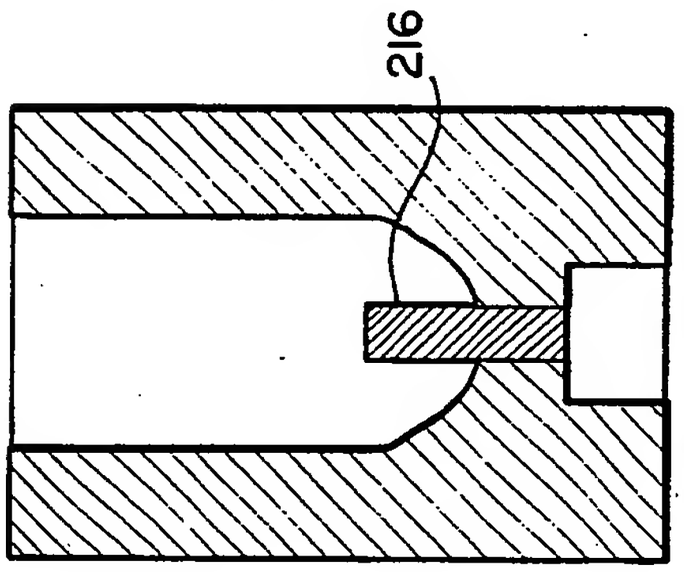

FIG. 29

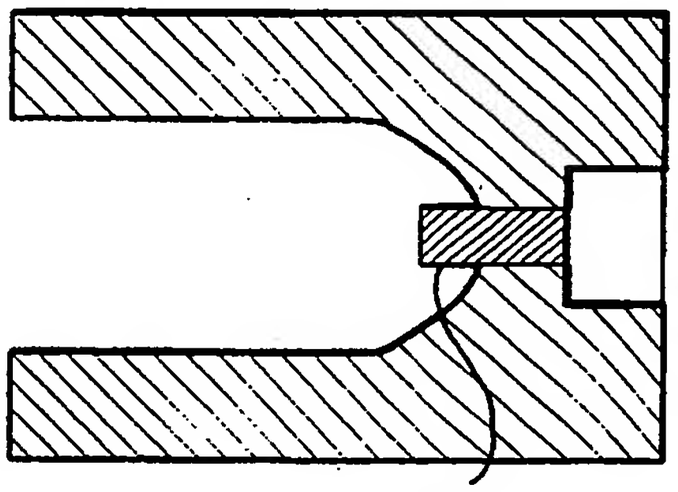

FIG. 28

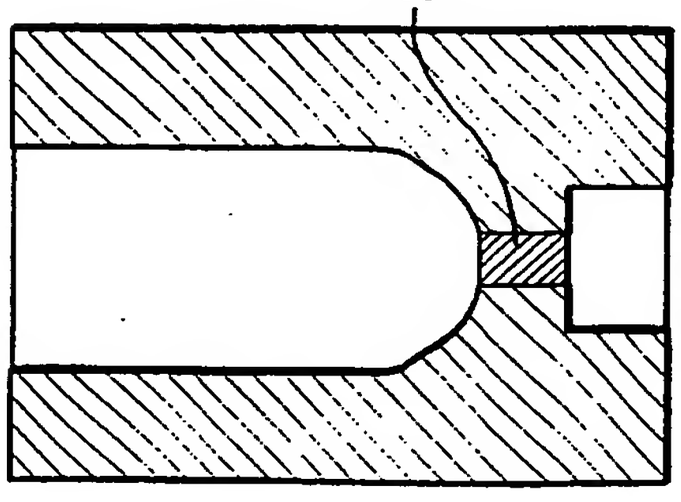

FIG. 27

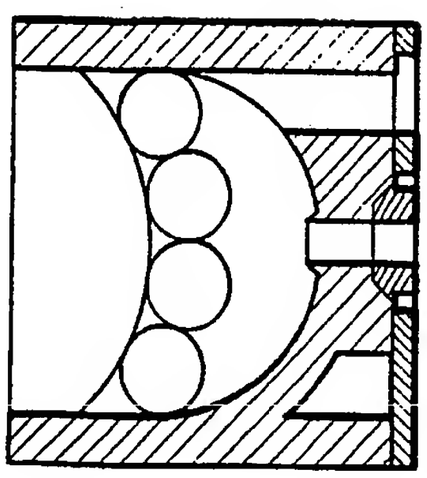

FIG. 25

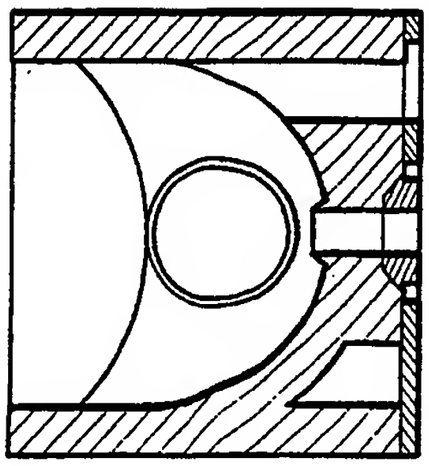

FIG. 24

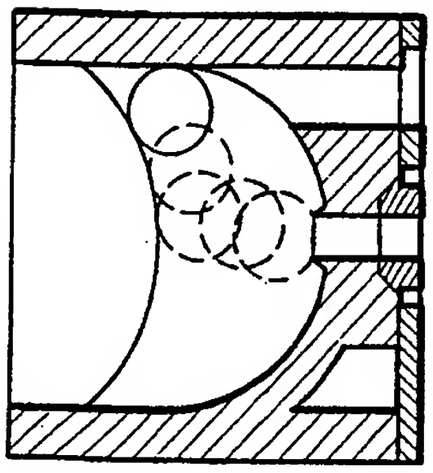

FIG. 23

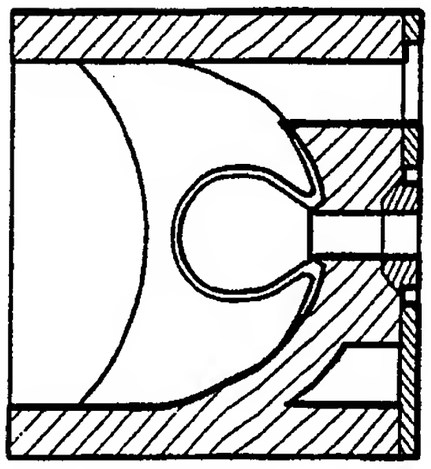

FIG. 22

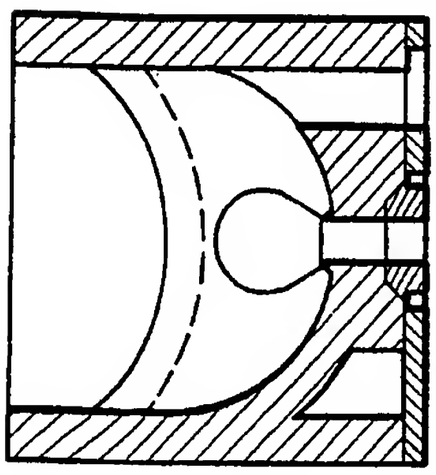

FIG. 21

INTERNATIONAL SEARCH REPORT

Inte. .tional Application No
PCT/US 99/07965

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 B01L3/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 35203 A (UNIVERSITY OF UTAH) 25 September 1997 (1997-09-25) cited in the application page 6, line 1 - line 26 page 7, paragraph 1 page 7, line 22 - page 8, line 4 page 8, line 11 - page 10, line 2 page 10, line 6 - line 15 page 13, line 6 - page 14, line 8 page 14, line 14 - line 32 page 15, line 6 - line 24 page 15, line 30 - page 16, line 21 page 16, line 30 - line 32 page 20, line 1 - page 21, line 10	1-6, 9-14,44, 45, 47-49, 51,53,54
Y	figures 1-4,6,7	7,20,30, 50,55

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
5 August 1999	13/08/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Thomas, R.M.

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INTERNATIONAL SEARCH REPORT

Int. .ational Application No
PCT/US 99/07965

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 223 219 A (SUBRAMANIAN) 29 June 1993 (1993-06-29) column 5, line 41 - column 6, line 7 column 6, line 12 - line 17 column 7, line 29 - line 32 column 9, line 18 - line 64 column 11, line 9 - line 21 column 12, line 11 - line 19 column 12, line 36 - line 43 column 13, last paragraph - column 18, line 3 column 18, line 15 - line 21 column 20, line 14 - line 20	21,23, 24,27, 32-37,43
Y	figures 1,2,5,10 ---	30,50
A	WO 97 02357 A (AFFYMETRIX) 23 January 1997 (1997-01-23) page 32, line 24 - page 33, line 7 page 44, line 6 - line 27 page 46, line 23 - page 47, line 23	1,2,5,9, 12,13, 21,23, 24,27, 36, 44-47, 49,51, 53,54
X	page 48, line 28 - page 49, line 9 page 49, line 35 - page 50, line 7 page 53, line 36 - page 54, line 11 page 58, line 3 - line 5 page 67, line 25 - line 30	19
Y	figures 5,12B ---	7,20,55
A	US 5 140 161 A (BIOTRACK) 18 August 1992 (1992-08-18) column 19, line 47 - column 20, line 7 column 20, line 53 - line 58 figures 2A,2B	1,5,9, 13,21, 36,44, 45,51
A	--- WO 94 06013 A (PACIFIC BIOTECH) 17 March 1994 (1994-03-17) page 6, line 7 - line 16 page 8, line 13 - line 37 page 11, line 8 - line 29 page 17, line 36 - page 18, line 2 figures 1,3,4	21,36
Y	--- EP 0 601 205 A (DAIKIN) 15 June 1994 (1994-06-15) page 3, paragraph 1 page 8, line 54 - line 57 figures 1,7	17,18
	--- -/--	

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INTERNATIONAL SEARCH REPORT

Int. ational Application No
PCT/US 99/07965

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category '	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 96 35940 A (CIBA-GEIGY) 14 November 1996 (1996-11-14) page 1, paragraph 1 page 24, penultimate paragraph page 29, line 1 - line 24 page 30, line 11 - line 13 figure 6 ----	17,18

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Int. ational Application No
PCT/US 99/07965

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		DE	3650610 D	15-05-1997
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INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte ..tional Application No
PCT/US 99/07965

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		CN 1092175 A	14-09-1994
		EP 0611450 A	24-08-1994
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EP 0601205 A	15-06-1994	DE 69323837 D	15-04-1999
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WO 9635940 A	14-11-1996	AU 5763296 A	29-11-1996
		CA 2219769 A	14-11-1996
		EP 0824684 A	25-02-1998
		PL 323257 A	16-03-1998
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[redacted]

[redacted]